BIRLA CENTRAL LIBRARY
PILANI (RAJASTHAN)

Call No 612·396
P415

Accession No 42054

STRUCTURAL CARBOHYDRATE CHEMISTRY

STRUCTURAL CARBOHYDRATE CHEMISTRY

BY

E. G. V. PERCIVAL
D.Sc., Ph.D., F.R.I.C.
READER IN CHEMISTRY, UNIVERSITY OF EDINBURGH

NEW YORK
PRENTICE-HALL, INC.
1950

PRENTICE-HALL CHEMISTRY SERIES
Wendell M. Latimer, Ph.D., *Editor*,
70 Fifth Avenue, New York

FIRST PUBLISHED BY FREDERICK MULLER, LTD.,
IN 1950
PRINTED IN GREAT BRITAIN BY
ROBERT MACLEHOSE AND CO. LTD., GLASGOW

PREFACE

THIS book is intended to be of service to students reading for an Honours degree in Chemistry, and to serve as a text-book in the later stages of preparation for ordinary and combined degrees, and for examinations in biochemistry and pharmaceutical chemistry. It is hoped also that research workers in both the academic and industrial fields will find some useful information within its pages.

When the original manuscript was completed early in 1948, the only text-book in English on the subject was *The Constitution of the Sugars*, and the advances made since the publication of Sir Norman Haworth's classic in 1928 appeared to be a sufficient justification for an attempt to bring the subject up to date. Owing to delays in publication it has been found possible to include new material from original work up to the early part of 1949.

I wish to acknowledge the debt I owe to my collaborators in research, and especially to those in whose laboratories I have had the good fortune to work, Professor W. Wardlaw, (the late) Professor H. Hibbert, Professor Sir Norman Haworth, Professor Kendall and Professor E. L. Hirst.

In the preparation of this book I have had the invaluable assistance of my wife, especially in the preparation of the formulae, the index and the manuscript, as well as in the reading of proofs and I wish to place on record my appreciation and thanks.

Finally I wish to thank the publishers for their assistance at every stage, and especially Dr. J. G. F. Miller who, indeed, persuaded me to write this book.

E. G. V. P.

September 1949

CONTENTS

CHAPTER PAGE

I Introduction. The General Properties, Configurations and Interrelationships of the Monosaccharides 1

General Properties of the Monosaccharides, 2; Stereoisomerism of the Sugars, 8; The Configuration of the Monosaccharides, 10; Some Important Transformations, 14; Ascent of the Series, 14; Epimerisation, 15; Descent of the Series, 17; The Interrelationships of the Various Sugars, 19; Fischer's Synthesis of the Hexoses, 23

II The Sugars as Ring Forms 25

The Methylglucosides, 25; The Isomeric Glucoses, 26; Mutarotation, 31; The Evaluation of the Ring Structures of Methylglycosides, 31; Haworth's Nomenclature for the Ring Forms, 43; The Equilibrium between Ring and Open Chain Forms, 46

III Characteristic Reactions of Sugars involving Hydroxyl Groups 50

Etherification, 50; Methylation, 50; Triphenylmethyl ethers, 52; Esterification, 53; Acetylation, 53; Other acylations, 53; Condensation with Acetone and Aldehydes, 54; Sugar Carbonates and the Crystalline Alkylfuranosides, 58; Sugar Halohydrins, 60; The Acetohalogenoses and their Reactions, 60; Glycals, 63; Deoxy Sugars, 63; Glycoseens, 64

IV Sugar Anhydrides and Amino Sugars 66

Glycosans, 66; 3, 6-Anhydrides: The Pentaphan or Hydrofuranol Ring, 67; Anhydrides of the Ethylene Oxide Type, 69; Their Formation and Fission, 73; The Amino Sugars, 74; D-Glucosamine, 74; Chondrosamine, 76

V Oligosaccharides 79

Disaccharides, 79; Reducing Disaccharides with 1, 4-Linkages, 80; Maltose, 80; Cellobiose, 82; Lactose, 83; Reducing Disaccharides with 1, 6-Linkages, 84; Gentiobiose, 84; Melibiose, 86; Primeverose, Vicianose, and Rutinose, 87; Reducing Disaccharides with 1, 3-Linkages, 88; Turanose, 88; Laminaribiose, 90; Non-Reducing Disaccharides, 90; Trehalose, 90; Sucrose, 91; The Synthesis of Disaccharides, 94; Enzymatic Syntheses, 98; Trisaccharides, 99; Gentianose, 99; Raffinose, 100; Melezitose, 100; Tetrasaccharide, Stachyose, 101

VI POLYSACCHARIDES 103

THE MAIN STRUCTURAL FEATURES OF CELLULOSE, STARCH, GLYCOGEN, INULIN AND XYLAN, 103; Cellulose, 104; Lichenin, 112; Starch, 112; Glycogen, 117; Inulin, 118; Xylan, 121

VII FURTHER STUDIES ON CELLULOSE, STARCH, GLYCOGEN AND XYLAN 123

CELLULOSE, 123; The Molecular Weight, 123; The Degradation during Methylation, 125; The Effect of Substitution on the Solubility of Cellulose Derivatives, 130; THE STARCH POLYSACCHARIDES, 133; AMYLOSE, 133; The Synthesis of Amylose by Enzymes, 135; AMYLOPECTIN, 138; The Synthesis of Amylopectin by Enzymes, 146; GLYCOGEN, 147; XYLAN, 148

VIII THE URONIC ACIDS AND THE POLYURONIDES 151

SIMPLE URONIC ACIDS, 151; ALDOBIURONIC ACIDS, KETOURONIC ACIDS, 154; POLYURONIDES, 156; Pectic Acid, 156; Alginic Acid, 158; Bacterial Polyuronides, 161; Plant Gums and Mucilages, 164; THE SEPARATION OF MIXTURES OF SUGARS FROM POLYSACCHARIDE HYDROLYSATES, by Chromatography etc., 167

IX NATURAL GLYCOSIDES 170

PHENOLIC GLYCOSIDES, 171; Hydroxy-anthraquinone Glycosides, 173; Hydroxy-coumarin Glycosides, 174; Hydroxyflavone Glycosides, 174; Xanthone Glycosides, 175; ANTHOCYANINS, 176; CYANOGENETIC GLYCOSIDES, 180; MUSTARD OIL GLYCOSIDES, 183; GLYCOSIDES DERIVED FROM *CYCLO*PENTENOPHENANTHRENE, 184; Saponins, 184; Cardiac Glycosides, 184; CEREBROSIDES etc. 185; NITROGEN GLYCOSIDES, Nucleotides and Nucleosides, 186; RIBOFLAVIN, 189

X OTHER IMPORTANT NATURAL PRODUCTS 191

POLYHYDRIC ALCOHOLS, 191; Erythritol, 191; Mannitol, 191; Sorbitol, 193; Dulcitol, 194; Heptahydric Alcohols, 195; CYCLIC POLYHYDRIC ALCOHOLS, 195; Inositols, 195; Quercitols, 199; DEOXY SUGARS, 199; ASCORBIC ACID, 201

XI SOME LESS FAMILIAR POLYSACCHARIDES 206

POLYGLUCOSANS, 206; Bacterial Dextrans, 206; Laminarin, 207; Yeast Glucan, 208; POLYFRUCTOSANS, 209; Levan, 209; POLYGALACTANS, 211; Snail Galactogen, 212; Pectin Galactan, 213; Galactocarolose, 213; POLYMANNANS, 214; Ivory Nut Mannan, 214; Yeast Mannan, 214; POLYSACCHARIDES CONTAINING NITROGEN, 215; Chitin, 215; POLYSACCHARIDES CONTAINING SULPHUR, 217; Carrageenin, 217; Agar, 217; POLYSACCHARIDES CONTAINING NITROGEN AND SULPHUR, 218

AUTHOR INDEX 220

SUBJECT INDEX 225

CHAPTER I

INTRODUCTION. THE GENERAL PROPERTIES, CONFIGURATIONS AND INTERRELATIONSHIPS OF THE MONOSACCHARIDES

Introduction

It is generally conceded that the carbohydrates, as a group, are compounds of the highest importance and interest, viewed from both the practical and the theoretical aspects. The process of photosynthesis by means of which all life on this planet is sustained, is concerned with the manufacture of carbohydrates in the plant from carbon dioxide and water, although we do not yet know the precise way in which this apparently facile transformation is brought about. Carbohydrates therefore represent a great storehouse of energy, either as foodstuffs for men and animals, or after transformations in the geological past, as coal and peat. Apart from these considerations and the special importance of carbohydrates in plant and animal biochemistry, the carbohydrates are of intrinsic interest as chemical individuals, and by their study chemistry, and especially stereochemistry, has been greatly enriched.

The term carbohydrate originated in the belief that naturally occurring compounds of this class for example glucose, $C_6H_{12}O_6$, sucrose $C_{12}H_{22}O_{11}$, starch $(C_6H_{10}O_5)_n$, could be represented formally as hydrates of carbon, that is $C_x(H_2O)_y$. This definition is too rigid, however, since the important deoxy sugars, for example rhamnose, $C_6H_{12}O_5$, the uronic acids and such compounds as ascorbic acid, would thereby be excluded and acetic acid and phloroglucinol would qualify for inclusion. Nevertheless the term carbohydrate remains in general use to describe those polyhydroxy compounds which reduce Fehling's solution either before or after hydrolysis with mineral acids.

The main divisions it is customary to make within the carbohydrate group are the *Monosaccharides* or simple sugars like glucose, xylose, fructose, the *Disaccharides* such as maltose, lactose, and sucrose, formed, as the name implies, by the union of two monosaccharide units, the *Trisaccharides* of which raffinose is an example, and the *Polysaccharides*, a group which includes starch, glycogen and cellulose, in each of which a large number of monosaccharide units are combined. The term *Polyuronide* denotes a polysaccharide containing uronic acid residues, and the term *Oligosaccharide* is used, especially on the Continent, to denote compound sugars containing between two and six simple sugar units, that is ranging from di- to hexa-saccharides.

It is necessary to subdivide the monosaccharide groups in such a way that the number of carbon atoms in the molecule is indicated in the name. Thus a hexose contains six, a pentose five, and a heptose seven carbon atoms in the molecule, and by using the prefix aldo-, or keto-, a distinction is made between those sugars which have a potential aldehydic group at one end of the chain, and those in which a keto group is fundamental to the structure. Thus glucose is described as an aldohexose and fructose a ketohexose. Finally, as will appear in the sequel, since sugars, whether simple or combined, normally exist in cyclic forms, and the type of ring may vary according to the circumstances of formation, it is frequently necessary to denote the precise ring system by a suffix, for example pyranose or furanose, to indicate a six- or a five-membered ring respectively, and so we have such expressions as glucopyranose, and xylofuranoside to define even more precisely the nature of the compound under consideration.

General Properties of the Monosaccharides

If we take the hexoses as typical examples we find that the simple sugars are generally colourless crystalline solids with a sweet taste. This latter property is evidently dependent on the constitution, for glucose is much sweeter than mannose and the ketohexose, fructose, is the sweetest of all the sugars; this explains the sweetness of honey which is a mixture of glucose and fructose. All the hexoses are readily soluble in water to give optically active solutions which readily reduce Fehling's solution and ammoniacal silver nitrate, char or caramelise very readily on heating or in the presence of concentrated sulphuric acid, and suffer extensive decomposition on heating with caustic alkalis.

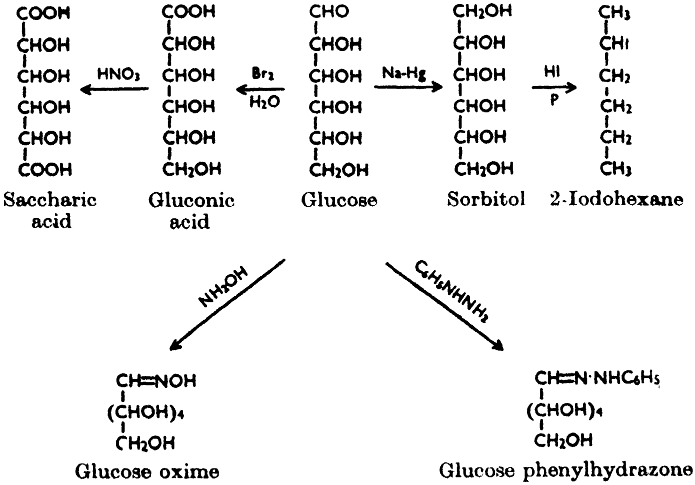
Emil Fischer* (1852-1919) applied his great gifts to a study of the monosaccharides and showed how they were interrelated. Although in some points of detail modifications have taken place since his time, Fischer laid the foundations of the present edifice of carbohydrate chemistry. Since Fischer's work on the sugars commenced (1884) with a study of the properties of glucose it is appropriate to make that sugar our starting point.

---

* *Untersuchungen über Kohlenhydrate und Fermente*, Berlin, 1909.

Glucose as a Pentahydroxy Aldehyde

That glucose can be represented as a pentahydroxy aldehyde is shown by the following facts. Reduction with sodium amalgam in faintly acid solution gives the hexahydric alcohol sorbitol. This gives a hexaacetate, and, on vigorous reduction yields 2-iodohexane, clearly showing that no branchings occur in the chain. Oxidation of glucose with bromine water gives gluconic acid and more vigorous oxidation with nitric acid yields saccharic acid, a tetrahydroxy dibasic acid. The fact, that on reduction glucose gives an alcohol and on mild oxidation a carboxylic acid without the loss of a carbon atom, is strong presumptive evidence that it contains an aldehydic group. This view is strengthened by the fact that glucose forms an oxime and a phenylhydrazone. Confirmatory evidence that an aldehydic group is present on the terminal position in glucose is furnished by the highly important reaction, discovered by H. Kiliani in 1886* wherein glucose unites with hydrocyanic acid to form a cyanhydrin (or more exactly two cyanhydrins), which on hydrolysis followed by reduction yield *n*-heptylic acid.

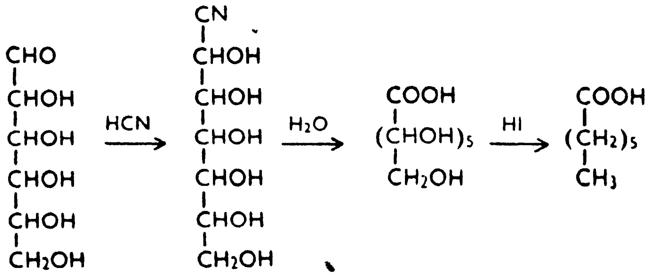


Saccharic acid — Gluconic acid — Glucose — Sorbitol — 2-Iodohexane

Glucose oxime — Glucose phenylhydrazone

---

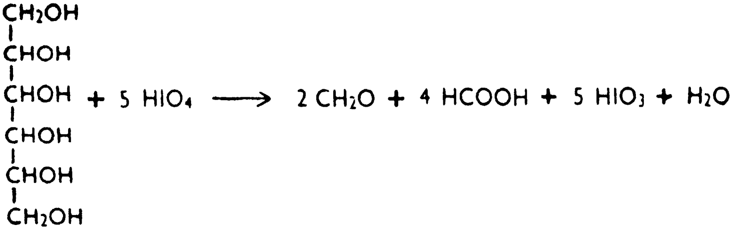
* *Ber.*, 1888, **21**, 915; 1886, **19**, 3029.



$$\begin{array}{c}CHO\\|\\CHOH\\|\\CHOH\\|\\CHOH\\|\\CHOH\\|\\CH_2OH\end{array}\xrightarrow{HCN}\begin{array}{c}CN\\|\\CHOH\\|\\CHOH\\|\\CHOH\\|\\CHOH\\|\\CHOH\\|\\CH_2OH\end{array}\xrightarrow{H_2O}\begin{array}{c}COOH\\|\\(CHOH)_5\\|\\CH_2OH\end{array}\xrightarrow{HI}\begin{array}{c}COOH\\|\\(CH_2)_5\\|\\CH_3\end{array}$$

Glucose cyanhydrin Glucoheptonic acid *n*-Heptylic acid

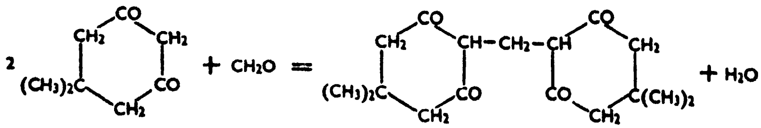
Perhaps the most direct confirmation of the above structure is furnished by the reaction, discovered by Malaprade*, and destined to have a very powerful influence on modern research in carbohydrate chemistry. Malaprade showed that periodic acid (HIO_4) smoothly oxidised α-glycols with the production of formic acid from a secondary alcohol group and formaldehyde from a primary alcohol residue. Thus the hexahydric alcohol, mannitol, consumed five molecules of periodic acid, readily estimated by titration, with the production of two molecules of formaldehyde and four molecules of formic acid.



$$\begin{array}{c}CH_2OH\\|\\CHOH\\|\\CHOH\\|\\CHOH\\|\\CHOH\\|\\CH_2OH\end{array} + 5\ HIO_4 \longrightarrow 2\ CH_2O + 4\ HCOOH + 5\ HIO_3 + H_2O$$

Mannitol

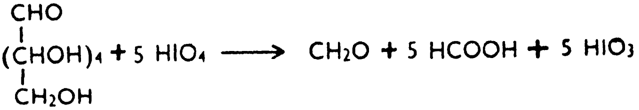
The formaldehyde is readily estimated as the crystalline condensation product with " dimedone " (5,5-dimethyl *cyclo*hexane 1,3-dione), and the formic acid by titration.



---

* *Bull. soc. chim.*, 1928, **43**, 683; 1934, (5), 1, 833.

When this facile reaction is applied to glucose* the products set out



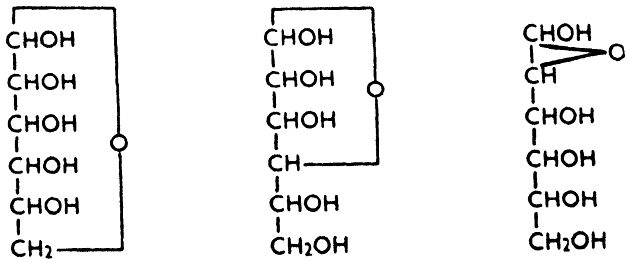
$$\begin{array}{l}\mathrm{CHO}\\ \quad|\\ (\mathrm{CHOH})_4\\ \quad|\\ \mathrm{CH_2OH}\end{array} + 5\ \mathrm{HIO_4} \longrightarrow \mathrm{CH_2O} + 5\ \mathrm{HCOOH} + 5\ \mathrm{HIO_3}$$

in the above equation are liberated.

The oxidation of sugars with lead tetraacetate, although not so convenient to apply, follows a somewhat similar course to that using periodic acid; lead tetraacetate, however, oxidises *cis* α-glycol groups preferentially† whereas periodic acid does not distinguish between *cis* and *trans* α-glycols.

Objections to the Representation of Glucose as a Free Aldehyde

Although the above evidence that glucose contains an aldehyde group may seem convincing, there are important facts which are in disagreement with this conclusion. As B. Tollens‡ pointed out, as far back as 1883, although glucose vigorously reduces Fehling's solution to cuprous oxide, and ammoniacal silver nitrate to metallic silver, the reducing power is far in excess of that which could be accounted for by a single aldehydic residue. Furthermore it may be stated that fructose, which as will be seen shortly is apparently a pentahydroxy ketone, reduces equally vigorously. The evidence in favour of an aldehydic structure based on these experiments is therefore suspect, and the suspicion is deepened by the fact that the colour is not restored to Schiff's reagent unless a specially sensitive reagent§ is used. To account for these facts Tollens suggested 1,4- and 1,6-oxide ring formulae; it should be added that a 1,2-oxide



Early cyclic formulae for glucose

---

* Fleury and Lange, *J. Pharm. Chim.*, 1933, **17**, 409.

† Hockett, Nickerson and Reeder, *J. Amer. Chem. Soc.*, 1944, **66**, 472.

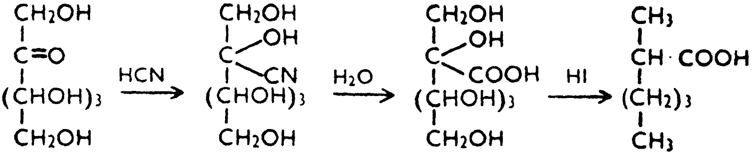
‡ *Ber.*, 1883, **16**, 921.

§ Tobie, *Ind. Eng. Chem.* (Anal.), 1942, **14**, 405.

ring had been suggested as early as 1870 by A. Colley.* It will be seen later that this matter of ring formation must be taken into account, but since sugar chemistry made remarkable progress at the end of the nineteenth century on the basis of the open-chain formulae and because certain stereochemical complications are necessarily introduced on ring closure, we shall continue for the present to consider only the acyclic formulae. There is in fact no real inconsistency in this for although the amount of the acyclic form may be very small, a proportion is always present, in aqueous solution, in equilibrium with the ring forms.

Fructose as a Pentahydroxy Ketone

Kiliani,† by methods similar to those already outlined for glucose, converted fructose into 2-methyl *n*-caproic acid, indicating that fructose is a 2-ketohexose.



Fructose

Fructose cyanhydrin

2-Methyl *n*-caproic acid

Sugar Osazones

Emil Fischer's introduction‡ of phenylhydrazine as a reagent in sugar chemistry is one of the milestones in the development of the subject. Sugars are notoriously difficult to crystallise and their melting points are frequently indefinite; in the phenylosazones, however, Fischer found a means of identifying the sugars by the isolation of bright yellow products usually of characteristic crystalline form. It may perhaps be mentioned in passing that although compared with the parent sugars the osazones are superior, most modern workers would agree that these compounds, since they are difficult to purify, are only used as a last resort for purposes of identification, and the osotriazoles described by C. S. Hudson obtained by oxidising the osazones with copper sulphate§ are to be preferred.

---

* *Compt. rend.*, 1870, **70**, 403.

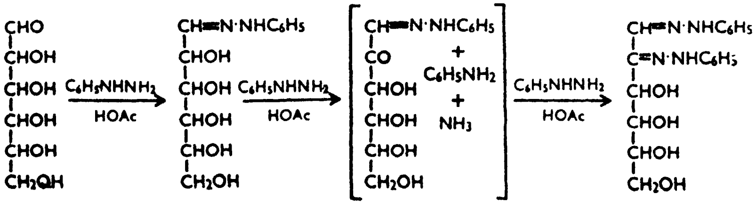
† *Ber.*, 1885, **18**, 3066.

‡ *Ber.*, 1884, **17**, 579.

§ Hann and Hudson, *J. Amer. Chem. Soc.*, 1944, **66**, 735; Hudson, *J. Org. Chem.*, 1944, **9**, 470.

Fischer's great success with the osazones, however, was in their use as intermediaries in transformation reactions and their employment in his stereochemical proofs.

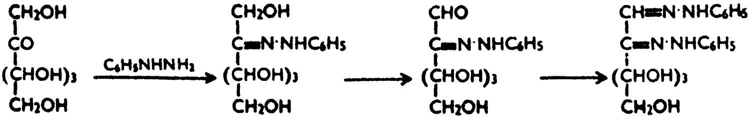
Taking glucose as the starting material, the first product of the reaction of phenylhydrazine acetate is glucose phenylhydrazone, this is then oxidised to a 2-keto derivative from which, by condensation with a further molecule of phenylhydrazine, glucose phenylosazone is obtained.



Glucose Glucose phenylhydrazone Glucose phenylosazone

There is still debate as to the precise mechanism of the reaction whereby oxidation of the secondary hydroxyl group on C_2 takes place; Fischer depicted it as due to the oxidising action of phenylhydrazine itself (which is converted to aniline and ammonia), but it is well known that the free base is normally a reducing agent. Kenner and Knight* have brought forward evidence that the phenylhydrazonium ion $[C_6H_5NHNH_3]^+$ is the effective oxidising agent, but other explanations have been advanced.†

Ketoses also form phenylosazones, in fact fructose and phenylhydrazine react much more rapidly than glucose and the reagent, although the *same* osazone is produced. In this case it is the primary alcoholic group on C_1 which is oxidised to give an aldehydohydrazone.



Fructose Glucose phenylosazone

The fact that glucose and fructose give the same osazone is important since it proves that, apart from the arrangements on carbon atoms 1 and 2, glucose and fructose must have identical structures.

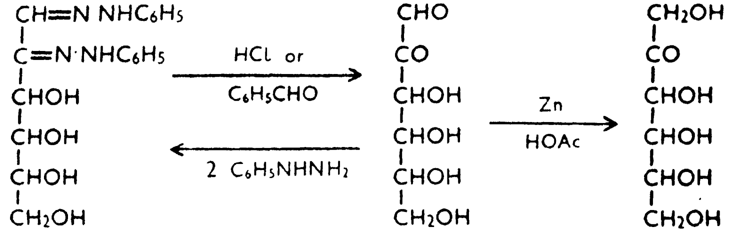
---

* *Ber.*, 1936, **69**, 341.

† See *Advances in Carbohydrate Chemistry*, 1948, **3**, 23, Academic Press, New York.

The osazone reaction is of course not confined to the use of phenylhydrazine itself; many substituted osazones, for example *p*-bromophenylosazones, tolylosazones, etc., have been prepared for identification purposes. C. Neuberg[*] introduced α-methylphenylhydrazine [$(C_6H_5N(CH_3).NH_2$] as a reagent for detecting ketoses, since it gives methylphenylosazones fairly readily with ketoses but not with aldoses, presumably because the base, or its hydrazonium ion, will oxidise a $\text{-}CH_2OH$ but not a $>CHOH$ group.

The osazones can be regarded as double hydrazones of an aldehydoketose, called an osone. Fischer prepared glucosone by removing the phenylhydrazino residues from glucosazone either by the use of concentrated hydrochloric acid, or a competing aldehyde such as benzaldehyde or acetaldehyde. The osone so obtained was found, as would be expected, to be a very reactive substance indeed, both reducing Fehling's solution in the cold and rapidly giving glucosazone with phenylhydrazine acetate at room temperature. By mild reduction the corresponding ketose, namely fructose, is obtained, the aldehyde group being reduced preferentially.[†] This reaction then allows us to transform an aldose into a ketose and, incidentally, emphasises the close relationship between glucose and fructose.



Glucose phenylosazone   Glucosone   Fructose

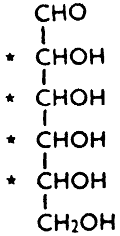
Stereoisomerism of the Sugars

It seems advisable at this point to consider the possibilities of stereoisomerism which is bound up with the important question of the optical activity of the sugars and their derivatives, for it is necessary to explain why there are, for example, sixteen different aldohexoses represented by the formula we have already used for glucose. An examination of the formula for an aldohexose reveals that we are dealing with a compound containing four different asymmetric carbon atoms; that is each of the atoms marked with an asterisk carries four different groups or atoms. At first sight to a beginner in stereochemistry, this fact may not be obvious; C_4 for example might be supposed to have H, OH and two -CHOH groups attached to it, but on further examination it is clear that the addenda are really H, OH, -CH(OH).CH_2OH and -CH(OH).CH(OH).CHO respectively. The number of optical isomers for *n* different asymmetric carbon atoms in a chain is 2^n, so that for an aldohexose there are sixteen isomers of which eight (the D-forms) will be mirror images of the other eight (the L-forms). For the aldopentoses, with three asymmetric carbon atoms, four D- and four L-forms exist and the same applies to the ketohexoses. At this point those studying the subject for the first time must be reminded that stereochemistry means chemistry in space, and that the carbon atoms are united through the tetrahedral angle of 109°28′. The two optical isomers of the simplest sugar, glycerose or glyceraldehyde, are depicted above, but it is a matter of some difficulty to represent the more complex sugars in this way. An aldopentose (D-xylose) might be written as shown (I) but such a formula is very inconvenient for ordinary use, and Fischer introduced the use of projection formulae[*] which, although they do not show all the essential features of the molecule, are much simpler for many purposes, the most serious drawback being that the tendency of the compounds under discussion to

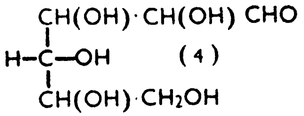
---

[*] *Ber.*, 1904, **37**, 4616.

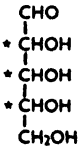
[†] Fischer, *Ber.*, 1889, **22**, 87.



$$\begin{array}{rl} & \mathrm{CHO} \\ & | \\ * & \mathrm{CHOH} \\ & | \\ * & \mathrm{CHOH} \\ & | \\ * & \mathrm{CHOH} \\ & | \\ * & \mathrm{CHOH} \\ & | \\ & \mathrm{CH_2OH} \end{array}$$

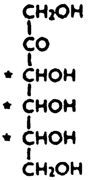


$$\begin{array}{l} \mathrm{CH(OH)\cdot CH(OH)\ CHO} \\ \quad | \\ \mathrm{H{-}C{-}OH} \qquad (4) \\ \quad | \\ \mathrm{CH(OH)\cdot CH_2OH} \end{array}$$



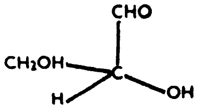
$$\begin{array}{rl} & \mathrm{CHO} \\ & | \\ * & \mathrm{CHOH} \\ & | \\ * & \mathrm{CHOH} \\ & | \\ * & \mathrm{CHOH} \\ & | \\ & \mathrm{CH_2OH} \end{array}$$

Aldopentose

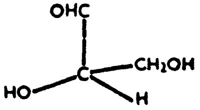


$$\begin{array}{rl} & \mathrm{CH_2OH} \\ & | \\ & \mathrm{CO} \\ & | \\ * & \mathrm{CHOH} \\ & | \\ * & \mathrm{CHOH} \\ & | \\ * & \mathrm{CHOH} \\ & | \\ & \mathrm{CH_2OH} \end{array}$$

Ketohexose



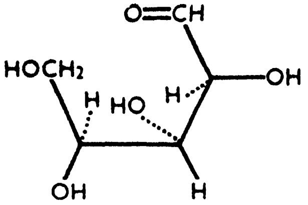
D-Glycerose



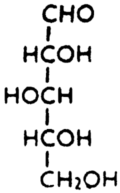
L-Glycerose

---

[*] Fischer, *Ber.*, 1891, **24**, 1836, 2683; Hudson, *J. Chem. Educ.*, 1941, **18**, 353.



(1)



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{HOCH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

D-Xylose

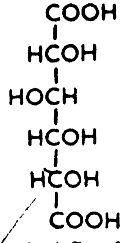
form five and six membered rings is obscured, although such possibilities of ring formation are obvious from a consideration of the extended formula (I) for xylose drawn above.

In writing the projection formulae certain conventions must be observed. The aldehyde (or hydroxyketo) group is put at the top of the formula, the loop of carbon atoms is theoretically straightened out, and it is arranged that the hydrogen atoms and hydroxyl groups on the intermediate carbon atoms are all projecting out from the plane of the paper. In other words the structure looked at roughly as an arc of a circle is viewed from the convex side of the arc. It is of course not allowable to remove a projection formula from the plane of the paper because there is then a danger of altering it into its enantiomorph, or mirror image form, but simple rotation in the plane of the paper is permissible. The example chosen represents one form of the aldopentose xylose. It now becomes necessary to explain why we have designated it as D-xylose.

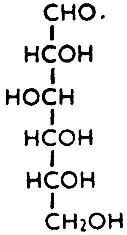
The Configuration of the Monosaccharides

Absolute and Relative Configuration

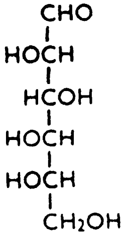
It is necessary to make clear at the outset that although we can determine the configurations of optically active compounds relative to one another, we cannot determine the actual disposition of the various groups in space. For example in the formulae on page 9 two mirror image forms of glycerose are set out, but we cannot lay down any absolute principle that will tell us which formula represents the isomer that will rotate the plane of polarised light to the right, and which to the left. Nevertheless it is possible by means of suitable reactions to convert an optically active substance (A) into another optically active product (B), and, in most instances to state with certainty that A and B have similar stereochemical arrangements in space. Naturally the configuration of A must be arbitrarily defined first. Fischer, by methods some of which will be mentioned later, was able to determine how the hydrogen atoms and hydroxyl groups were disposed in glucose and he chose the formulae below as representing the two isomeric glucoses. As explained above the choice between the two formulae was entirely arbitrary, but it was clearly essential to have a fixed point to which all the monosaccharides could be configuratively related. By various transformations and interconversions Fischer related other hexoses and the pentoses to the above structures through the saccharic acids, and all those related to dextrose (named above D(+)-glucose) were grouped in the D-series, irrespective of the actual sign of the optical rotation, and their mirror images in the L-series. This type of convention is *quite independent of the actual sign of the rotation*, which is indicated by a (+)- or (–)-sign as above or by the words *dextro* or *laevo*. For example D-glucose can be converted into the ketose D-fructose, but this sugar as the common name, laevulose, indicates is laevorotatory. Unfortunately Fischer's system led to anomalies with certain sugars such as xylose and gulose, and this led to its replacement by a much more logical system due to M. A. Rosanoff.*


$\begin{array}{c}\mathrm{COOH}\\ | \\ \mathrm{HCOH}\\ | \\ \mathrm{HOCH}\\ | \\ \mathrm{HCOH}\\ | \\ \mathrm{HCOH}\\ | \\ \mathrm{COOH}\end{array}$

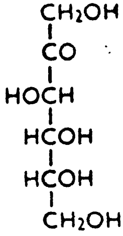
D(+)-Saccharic acid


$\begin{array}{c}\mathrm{CHO}\\ | \\ \mathrm{HCOH}\\ | \\ \mathrm{HOCH}\\ | \\ \mathrm{HCOH}\\ | \\ \mathrm{HCOH}\\ | \\ \mathrm{CH_2OH}\end{array}$

D(+)-Glucose

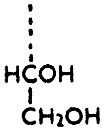

$\begin{array}{c}\mathrm{CHO}\\ | \\ \mathrm{HOCH}\\ | \\ \mathrm{HCOH}\\ | \\ \mathrm{HOCH}\\ | \\ \mathrm{HOCH}\\ | \\ \mathrm{CH_2OH}\end{array}$

L(–)-Glucose

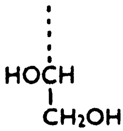

$\begin{array}{c}\mathrm{CH_2OH}\\ | \\ \mathrm{CO}\\ | \\ \mathrm{HOCH}\\ | \\ \mathrm{HCOH}\\ | \\ \mathrm{HCOH}\\ | \\ \mathrm{CH_2OH}\end{array}$

D(–)-Fructose

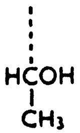
Rosanoff chose as his criterion the direction of the hydroxyl group on the penultimate carbon atom; if this pointed to the right in the projection formula where the aldehyde or the hydroxyketo group is written at the head, he assigned the compound to the D-series. In other words Rosanoff chose as his fixed point one of the two possible forms of glyceraldehyde (glycerose) and represented the D-form as


$\begin{array}{c}\vdots\\ \mathrm{HCOH}\\ | \\ \mathrm{CH_2OH}\end{array}$

D-series


$\begin{array}{c}\vdots\\ \mathrm{HOCH}\\ | \\ \mathrm{CH_2OH}\end{array}$

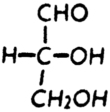
L-series


$\begin{array}{c}\vdots\\ \mathrm{HCOH}\\ | \\ \mathrm{CH_3}\end{array}$

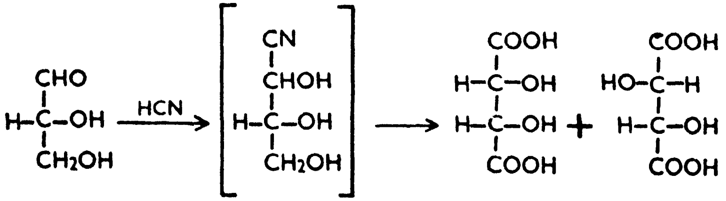
D-series

---

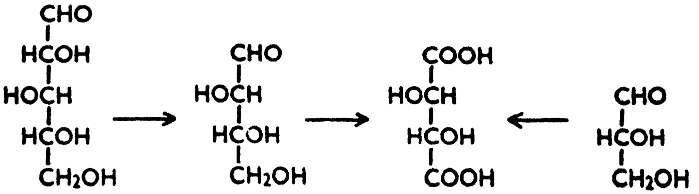
* *J. Amer. Chem. Soc.*, 1906, 28, 114.



and regarded all other sugars of the D-series as built up, in theory, from this substance. Although not accomplished in practice throughout the series such a process is rendered possible, without interfering with the original asymmetric carbon atom, by the Kiliani synthesis (see p. 14). It must be emphasised that the Rosanoff representation is merely a convention, independent of the actual sign of rotation of the sugar in question or, indeed, of the sign of rotation of the glycerose from which (+)-glucose could theoretically be prepared; in fact glycerose had not been resolved at that time. In 1917, however, Wohl and Momber[*] showed the relationship of (+)-glycerose to (−)-tartaric acid, since a mixture of *meso-* and (−)-tartaric acids were obtained on the hydrolysis and subsequent oxidation of the cyanhydrin of (+)-glycerose. The mixture of acids is obtained because the aldehyde group is symmetrical and we would not expect the new hydroxyl group to occupy an exclusive position on the new asymmetric carbon atom. Now Hockett[†] has shown that the tetrose D(+)-threose gives (−)-tartaric acid on oxidation, while threose itself is obtained by the degradation of D(+)-xylose, the relationship of which to D(+)-glucose and the other sugars is well



D(+)-Glycerose   *Meso*tartaric acid   D(−)-Tartaric acid



D-Xylose   D-Threose   D(−)-Tartaric acid   D(+)-Glycerose known and will be dealt with later in this chapter. There is thus a direct relationship between D(+)-glycerose and the D-series of sugars, *but the value of Rosanoff's contribution as a unifying influence is quite independent of this fact.*

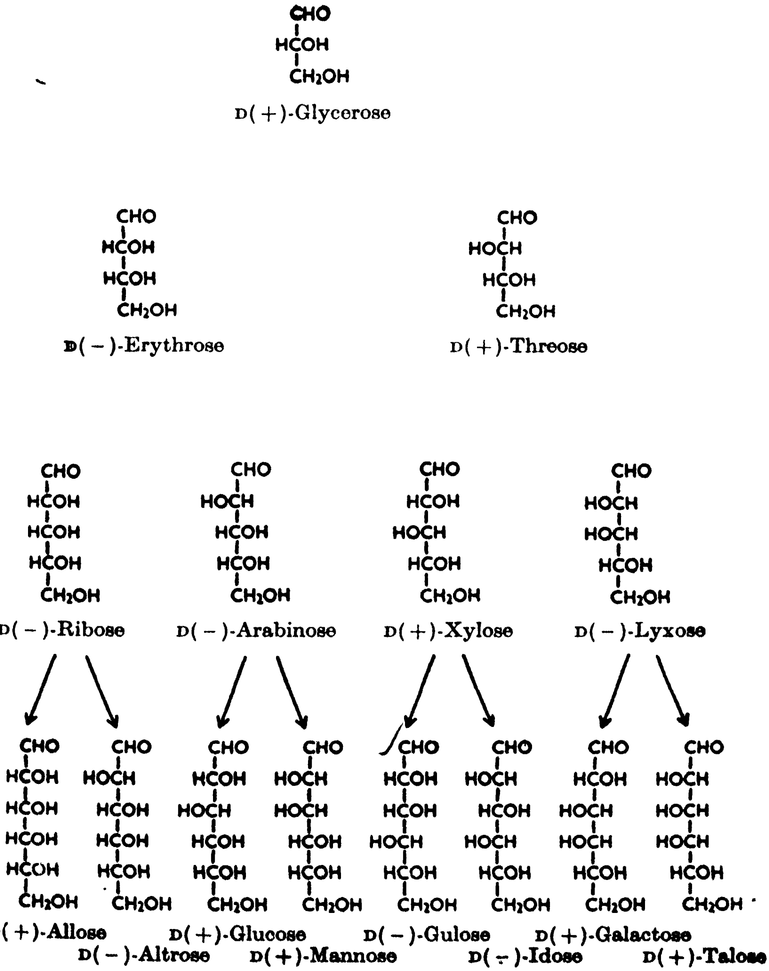
---

[*] *Ber.*, 1917, **50**, 455.

[†] *J. Amer. Chem. Soc.*, 1935, **57**, 2260.

The annexed diagram shows the relationship of D-glycerose to the D-trioses, -tetroses, -pentoses and -hexoses. Before considering evidence why these sugars should be represented thus, some important general reactions will be dealt with.

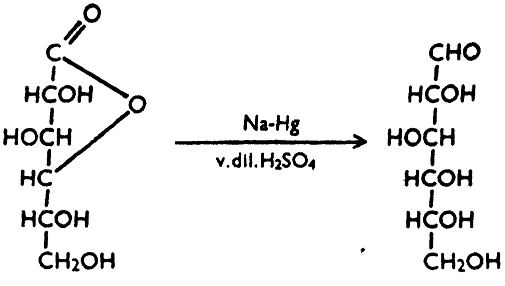
The D-Series of Sugars



Some Important Transformations

Conversion of the Lactones of Sugar Acids into Aldoses

Fischer discovered that when the γ-lactone of a sugar acid, for example γ-D-gluconolactone, is reduced by sodium amalgam in the presence of very dilute sulphuric acid, the corresponding aldose is produced. This reaction was of great value in conjunction with Kiliani's method for ascending the sugar series.

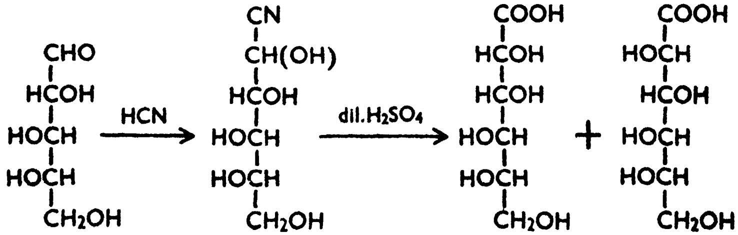


γ-D-Gluconolactone D-Glucose

Ascent of the Aldose Series

The Kiliani Reaction

The fact that aldoses (and ketoses) form cyanhydrins has already been mentioned (page 12) in connexion with the relationship between D(+)-glycerose and D(−)-tartaric acid. The reaction is of general application and has been of great importance in deciding the stereochemical configuration of the hexoses and in the synthesis of higher carbon sugars such as heptoses, octoses and so on. For example L-arabinose on conversion to the cyanhydrin and hydrolysis gives a mixture of L-mannonic and L-gluconic acids. The acids may be separated by the preparation of their crystalline phenylhydrazides,



L-Arabinose **L-Mannonic acid** **L-Gluconic acid**

fractional crystallisation and subsequent decomposition, and, after heating to form the γ-lactones and reduction with sodium amalgam in acidulated water, the sugars L-mannose and L-glucose are obtained.

The application of this reaction to any aldose, leads theoretically to the production of two new aldoses higher in the series, although it does not follow that both forms will be present in sufficient quantity to be separated readily.

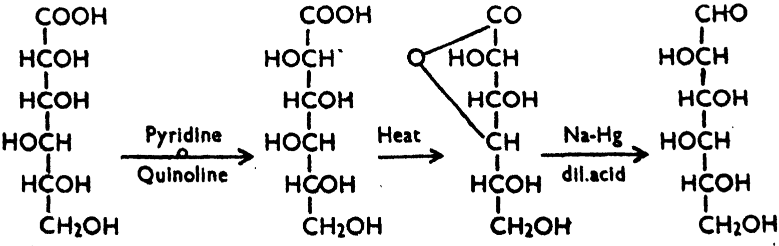
Another useful method of ascending the aldose series has been described by J. C. Sowden and H. O. L. Fischer.[*] An appropriately substituted aldose, e.g. 2, 4-benzylidene-L-xylose, is caused to react with nitromethane in the presence of sodium methoxide. This produces a C-nitro alcohol which, after conversion to the *aci*-sodium salt and treatment with moderately concentrated sulphuric acid, is transformed into an aldose containing one more carbon atom, namely L-gulose in the above case.

$$\begin{aligned} \mathrm{RCHO} + \mathrm{CH_3NO_2} \rightarrow \mathrm{RCHOH.CH_2NO_2} \rightarrow \mathrm{RCHOH.CH{=}NOONa} \\ \rightarrow \mathrm{RCHOH.CHO} \end{aligned}$$

In the examples studied so far, only one aldose is produced, no L-idose is isolated from the above reaction.

The Epimerisation of Aldonic Acids

When two sugars or their derivatives, such as the sugar acids, differ only in the configuration of the carbon atom adjacent to the reducing group they are said to be epimeric. We have already met with an example of this in the products of the previous reaction. By heating an aldonic acid in aqueous pyridine or quinoline it is possible to convert it partially into the epimeric acid. Thus D-gulonic acid may be transformed into D-idonic acid.[†] Following a separation by appropriate means the pure epimeric acid, after con-



D-Gulonic acid D-Idonic acid γ-D-Idonolactone D-Idose version into the γ-lactone, may be converted into the corresponding sugar, in this case D-idose.

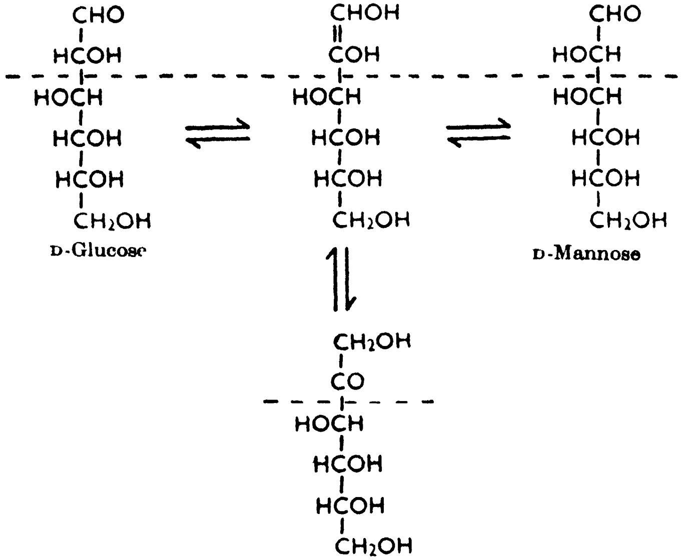
---

[*] *J. Amer. Chem. Soc.*, 1945, 67, 1713.

[†] Fischer and Fay, *Ber.*, 1895, 28, 1975.

The Lobry de Bruyn Transformation

In 1895 Lobry de Bruyn and Alberda van Eckenstein* discovered that an aqueous solution of glucose when made feebly alkaline with lime-water, contained, after standing for some time, the epimeric sugar, mannose, and the 2-ketohexose, fructose. This reaction which is of general application is due to the production of enolic forms in the presence of hydroxyl ions, followed by a rearrangement as follows.



D-Glucose

D-Mannose

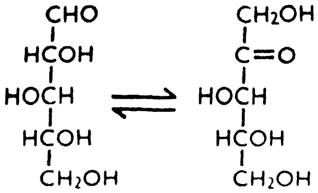
D-Fructose

This transformation serves to show the close relationship between glucose, mannose, and fructose; the three sugars are identical below the dotted line in the formula, which explains why they all give the same osazone. When the hydroxyl group on C_2 is substituted, however, treatment with aqueous alkali does not give a ketose since the substituent group cannot migrate to C_1 under these conditions, although the epimeric aldose is formed. Thus 2,3,4-trimethyl xylose gives 2,3,4-trimethyl lyxose,† and 2,3,4,6-tetramethyl glucose gives 2,3,4,6-tetramethyl mannose.

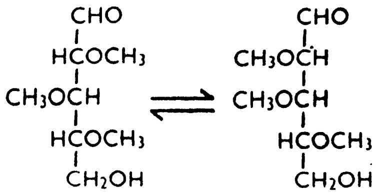
---

* *Rec. trav. chim.*, 1895, **14**, 203.

† M. L. Wolfrom and W. L. Lewis, *J. Amer. Chem. Soc.*, 1928, **50**, 837; C. E. Gross with W. L. Lewis, *ibid.*, 1931, **53**, 2772.



D-Xylose D-Xyloketose



2,3,4-Trimethyl D-xylose 2,3,4,-Trimethyl D-lyxose

When organic bases are used, for example pyridine or quinoline aldoses may be transformed into ketoses. Thus D-xylose gives D-xyloketose on heating with pyridine.

An important method for the preparation of ketoses has been introduced by Wolfrom and his co-workers.* The acetylated acyl chlorides of aldonic acids (aldonyl chlorides) react with diazomethane to give diazomethyl ketones which, on hydrolysis and subsequent careful deacetylation, yield ketoses.

$$R.COCl + CH_2N_2 \rightarrow RCOCHN_2 \rightarrow RCOCH_2OH$$

In this way L-fructose has been prepared from L-arabonic acid.

The action of dilute alkali on reducing sugars can cause even more deep seated changes than those discussed above, for example the enolisation is not confined to the production of the 1,2-dienol, but the 2,3-dienol is also produced together with degradation products such as glyceraldehyde. With more concentrated alkali, rearrangements take place with the production of saccharinic acids,† for example $HOOC.CH(OH).CH_2(CHOH)_2.CH_2OH$.

The Descent of the Aldose Series

Several methods are available for degrading sugars; this is very important for the determination of configurations.

The Method of Wohl‡ (Zemplén modification)§

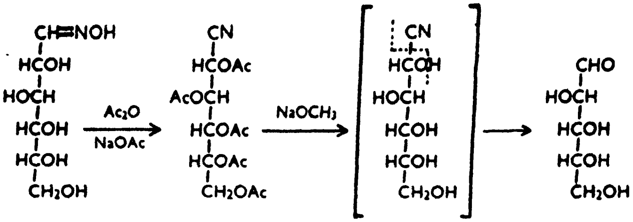
When a sugar oxime is acetylated, an acetylated cyanide is produced which on catalytic deacetylation in chloroform solution with sodium in methanol (see page 82) also loses the elements of hydrocyanic acid and a lower aldose is produced.

---

* Wolfrom and Thompson, *J. Amer. Chem. Soc.*, 1946, **68**, 791.

† J. U. Nef, *Annalen*, 1907, **357**, 294; 1910, **376**, 1; 1914, **403**, 204.

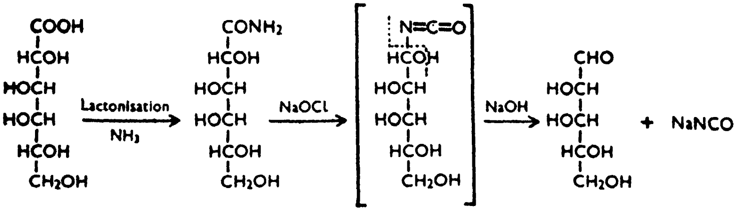
‡ *Ber.*, 1893, **26**, 730. § Zemplén and Kiss, *Ber.*, 1927, **60**, 165.



D-Glucose oxime D-Arabinose

*Weerman Degradation**

In this case the aldonic acid is converted into the amide by treating either the ester or the lactone with ammonia. Treatment with alkaline sodium hypochlorite gives as an intermediate an *iso*cyanate, which loses *iso*cyanic acid under the influence of the alkali. In the following example, which is of course a variation of the Hofmann reaction, D-lyxose is obtained from D-galactonic acid. Incidentally this reaction provides a very useful diagnostic test for the presence of a free α-hydroxy group, on which it is dependent. Sodium *iso*cyanate is readily identified since on treatment with semicarbazide hydrochloride and sodium acetate it condenses to yield the insoluble easily identifiable product, hydrazodicarbonamide.



D-Galactonic acid D-Lyxose

$$NH_2CONHNH_2 + OCNH = NH_2CONHNHCONH_2$$

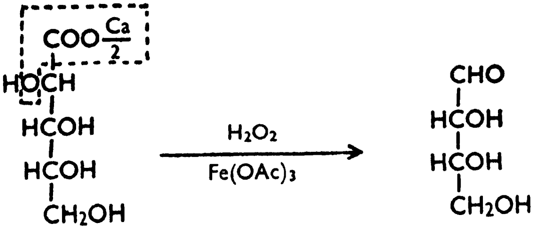
Ruff Degradation

By the oxidation of the calcium salt of an aldonic acid with hydrogen peroxide in the presence of ferric acetate, Ruff† succeeded in descending the series. Thus from calcium D-arabonate, D-erythrose was obtained.

---

* *Rec. trav. chim.*, 1917, **37**, 16.

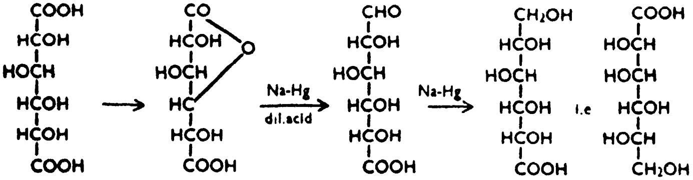
† *Ber.*, 1899, **32**, 553, 3672.



Calcium D-arabonate D-Erythrose

Fischer's Conversion of D-Glucose into L-Gulose*

On oxidation with nitric acid, D-glucose is converted into D-saccharic acid, the γ-lactone of which on reduction with sodium amalgam and acidulated water gives D-glucuronic acid. Further reduction with sodium amalgam and water yields an aldonic acid which belongs to the L-series and is L-gulonic acid. On conversion to the γ-lactone, followed by reduction L-gulose is obtained. On account of the fact that it was prepared from D-glucose (dextrose) Fischer considered this substance to belong to the D-series, a decision which caused subsequent confusion.



D-Saccharic acid D-Saccharo-lactone D-Glucuronic acid - L-Gulonic acid

The Interrelationships of the Various Sugars

With the use of such reactions and transformations as those recently described, the interrelationships of the various sugars can be demonstrated. We have already indicated, for example, the value of the comparisons of the properties of osazones prepared from different sugars in deciding whether the stereochemical arrangements of hydrogen atoms and hydroxyl groups on the carbon atoms after the first two are identical or not. Thus D-glucose, D-mannose, and D-fructose give the same osazone, a compound with properties quite different from galactose phenylosazone. It must also be borne in mind that, of the aldohexoses only D- and L-galactose, D-glucose and D-mannose, and of the ketohexoses only D-fructose, L-sorbose and

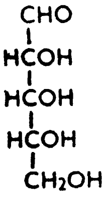
---

* Fischer and Piloty, *Ber.*, 1891, **24**, 521.

D-tagatose occur in natural products; the remainder are synthetic, being prepared by such methods as the epimerisation of the sugar acids or by ascending the series or, in the case of ketoses, from the osazones *via* the osones. It is obvious that once the stereochemical arrangements in a few key sugars are established it is possible to deduce the relative configurations of the rest, and Fischer did this for the twelve aldohexoses and the two ketohexoses known at that time. The arrangements deduced by Fischer were absolutely correct, although as mentioned earlier, in some instances he described compounds now regarded as belonging to the D-series as of the L-series and *vice versa.*

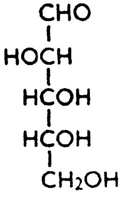
The Pentoses and Tetroses

It is necessary first of all to deduce the relative configuration of the pentoses. The four possibilities (in the D-series) are given below.



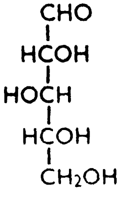
$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

(I)



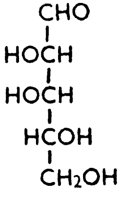
$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{HOCH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

(II)



$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HOCH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

(III)



$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{HOCH}\\ |\\ \mathrm{HOCH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

(IV)

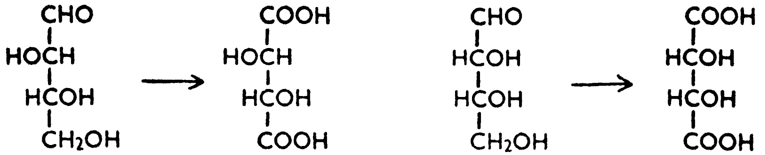
Identical osazones are given by two pairs of pentoses, namely ribose and arabinose, and xylose and lyxose, so that these pairs are (I) and (II); (III) and (IV). By degradation Ruff[*] prepared from D-xylose the tetrose, D(+)-threose which as already stated (page 12) gave (−)-tartaric acid on oxidation with nitric acid. The pentose D-arabinose, on the other hand gave D-erythrose[†] on degradation which on reduction gave the optically inactive, naturally occurring alcohol, *meso*-erythritol, and this on oxidation[‡] was known to give *meso*tartaric acid. The only possible formulae for the two D-aldotetroses are therefore those given above, and it follows that D-xylose must be either (III) or (IV) and arabinose either (I) or (II). Xylose on oxidation with nitric acid gives an inactive (*meso*) trihydroxyglutaric acid. Only (III) could afford such a compound since the product contains a plane of symmetry, therefore D-xylose is (III) and D-lyxose must be (IV). D-Arabinose on oxidation gives an

---

[*] Ruff and Kohn, *Ber.*, 1901, **34**, 1370.

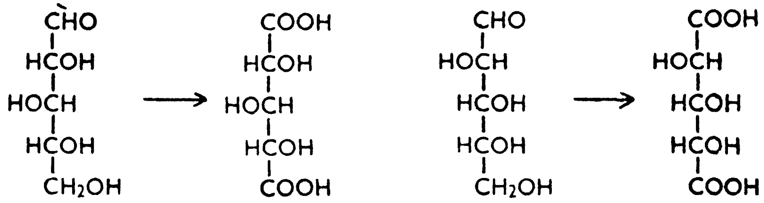
[†] Wohl, *Ber.*, 1893, **26**, 743. Ruff, *Ber.*, 1899, **32**, 3672.

[‡] S. Przybytek, *Ber.*, 1881, **14**, 1202; 1884, **17**, 1412.



D(+)-Threose D(−)-Tartaric acid D-Erythrose *Meso*tartaric acid

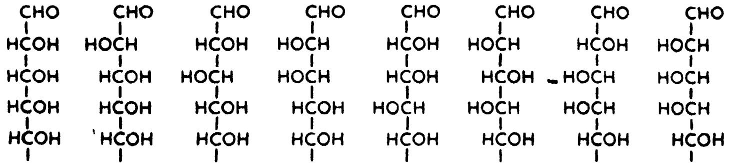
active trihydroxyglutaric acid, so that this pentose cannot be (I), the oxidation product of which would have a plane of symmetry, and must necessarily be (II). D-Ribose must therefore be (I), and this is confirmed because on reduction[*] it is converted into the inactive (*meso*) pentahydric alcohol, adonitol, which occurs in the sap of certain plants.



(III) i-*xylo*trihydroxyglutaric acid (II) D-*arabo*trihydroxyglutaric acid

The D-Series of Aldohexoses

The eight possibilities are set out below:



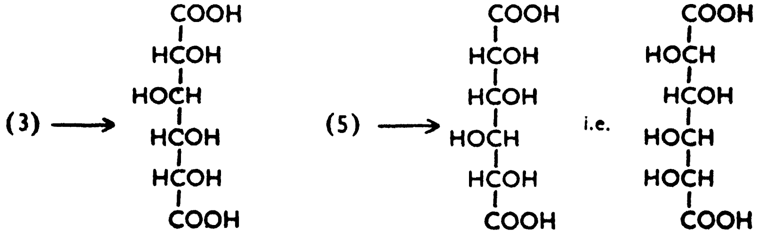
(1) (2) (3) (4) (5) (6) (7) (8)

The two aldohexoses, allose and altrose, were prepared in 1910 by Levene and Jacobs[†] by ascent of the series from D-ribose. These sugars must therefore be the pair (1), (2). Since altrose on oxidation gives the previously known D-talomucic acid whereas allose gives an inactive hexahydric alcohol on reduction, allose therefore must be (1) and altrose (2). By the degradation of D-glucose, D-arabinose is obtained, glucose and mannose give the same osazone and this epimeric pair must therefore be (3), (4). Fischer decided between them by examining the dibasic (saccharic) acids obtained on oxidation with nitric acid. Another hexose, L-gulose (see page 19) on oxidation gave the same saccharic acid as glucose. An examination of the formulae (3) and (4) reveals that by reasons of symmetry, the acid (or its enantiomorph) obtained on oxidising (4) could not be derived from any of the other hexoses. There is, however, *one* other formulation, namely (5) which would yield on oxidation a saccharic acid which would be the enantiomorph of that prepared from (3). Thus (3) must be D-glucose, (4) D-mannose and (5) D-gulose. Idose and gulose give the same osazone, therefore idose is (6). The relationship of D-lyxose to D-galactose has been indicated on page 18 for by the degradation of galactose lyxose is obtained. This would agree with the assignment of either (7) or (8) to D-galactose. Oxidation of galactose gives the well-known dibasic acid, mucic acid, which is a *meso* form. D-galactose must therefore be (7) since the dibasic acid derived from (7) as distinct from that prepared from (8) would have a plane of symmetry, and (8) is the epimeric sugar D-talose which gives D-galactosazone on treatment with phenylhydrazine acetate.

---

[*] Fischer, *Ber.*, 1893, **26**, 633.

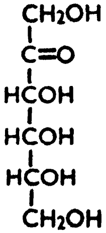
[†] *Ber.*, 1910, **43**, 3141.



$$(3) \longrightarrow \begin{array}{c} \mathrm{COOH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{HOCH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{COOH} \end{array} \qquad (5) \longrightarrow \begin{array}{c} \mathrm{COOH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{HOCH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{COOH} \end{array} \quad \text{i.e.} \quad \begin{array}{c} \mathrm{COOH} \\ \mid \\ \mathrm{HOCH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{HOCH} \\ \mid \\ \mathrm{HOCH} \\ \mid \\ \mathrm{COOH} \end{array}$$

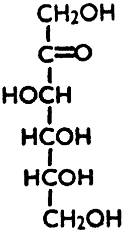
The D-Series of 2-Ketohexoses

The four possible 2-ketohexoses are formulated below. (B) is recognised at once as D-fructose because treatment with phenylhydrazine gives glucosazone. The osazone of L-gulose is the same



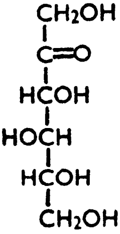
$$\begin{array}{c} \mathrm{CH_2OH} \\ \mid \\ \mathrm{C{=}O} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{CH_2OH} \end{array}$$

(A)
D-Psicose
D-Allulose



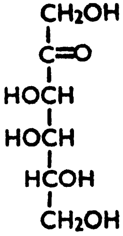
$$\begin{array}{c} \mathrm{CH_2OH} \\ \mid \\ \mathrm{C{=}O} \\ \mid \\ \mathrm{HOCH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{CH_2OH} \end{array}$$

(B)
D-Fructose



$$\begin{array}{c} \mathrm{CH_2OH} \\ \mid \\ \mathrm{C{=}O} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{HOCH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{CH_2OH} \end{array}$$

(C)
D-Sorbose



$$\begin{array}{c} \mathrm{CH_2OH} \\ \mid \\ \mathrm{C{=}O} \\ \mid \\ \mathrm{HOCH} \\ \mid \\ \mathrm{HOCH} \\ \mid \\ \mathrm{HCOH} \\ \mid \\ \mathrm{CH_2OH} \end{array}$$

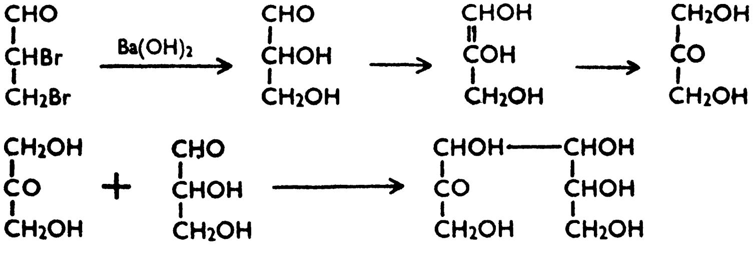
(D)
D-Tagatose

as the osazone of natural L-sorbose, so that (C) represents D-sorbose. When D-allose is boiled with anhydrous pyridine D-psicose is produced, so that this ketose must be (A), and the remaining ketose, D-tagatose is prepared from D-galactose by the action of aqueous alkali and gives the same osazone as galactose and is consequently(D). D-Tagatose has recently been found among the hydrolysis products of *sterculia setigera* gum.*

By methods such as those outlined above, although not necessarily in the first instance by the actual methods described, since an attempt has been made to give as simple an account as possible, the relative stereochemical relationships of the monosaccharides have been deduced.

Fischer's Syntheses of Hexoses

One of the most outstanding achievements of organic chemistry was the synthesis, virtually from the elements, of a large number of the hexose sugars by Emil Fischer.† He first prepared a mixture of optically inactive hexoses, which he called acrose, either by the action of mild alkali on acrolein dibromide or by the action of bromine and sodium carbonate on glycerol. The most plausible explanation of the condensation is represented diagrammatically below,



$$CHO{\cdot}CHBr{\cdot}CH_2Br \xrightarrow{Ba(OH)_2} CHO{\cdot}CHOH{\cdot}CH_2OH \longrightarrow CHOH{=}COH{\cdot}CH_2OH \longrightarrow CH_2OH{\cdot}CO{\cdot}CH_2OH$$

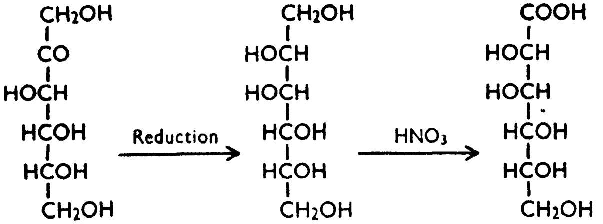
$$CH_2OH{\cdot}CO{\cdot}CH_2OH + CHO{\cdot}CHOH{\cdot}CH_2OH \longrightarrow CH_2OH{\cdot}CO{\cdot}CHOH{-}CHOH{\cdot}CHOH{\cdot}CH_2OH$$

depending essentially on an aldol condensation between glyceraldehyde and dihydroxyacetone. Fischer also obtained acrose from the products of treating formaldehyde with alkali (Loew 1886), a development of an earlier experiment using paraformaldehyde (Butlerow 1861), and in this instance too a series of aldol condensations is involved. The acrose isolated by these methods was by no means a pure chemical individual, but by treatment with phenylhydrazine DL-glucosazone was isolated. Conversion to the osone and reduction gave DL-fructose, which on fermentation with yeast gave L-fructose, the D-form being destroyed. On reduction the DL-fructose gave the corresponding hexahydric alcohol, DL-mannitol, and by cautious oxidation DL-mannonic acid was isolated. In this connexion it is worthy of note that because of its symmetry, the conversion of either of the primary alcohol groups in mannitol to carboxyl gives the same product. DL-Mannonic acid was resolved

---

* Hirst, Hough and J. K. N. Jones, *Nature*, 1949, **163**, 177.

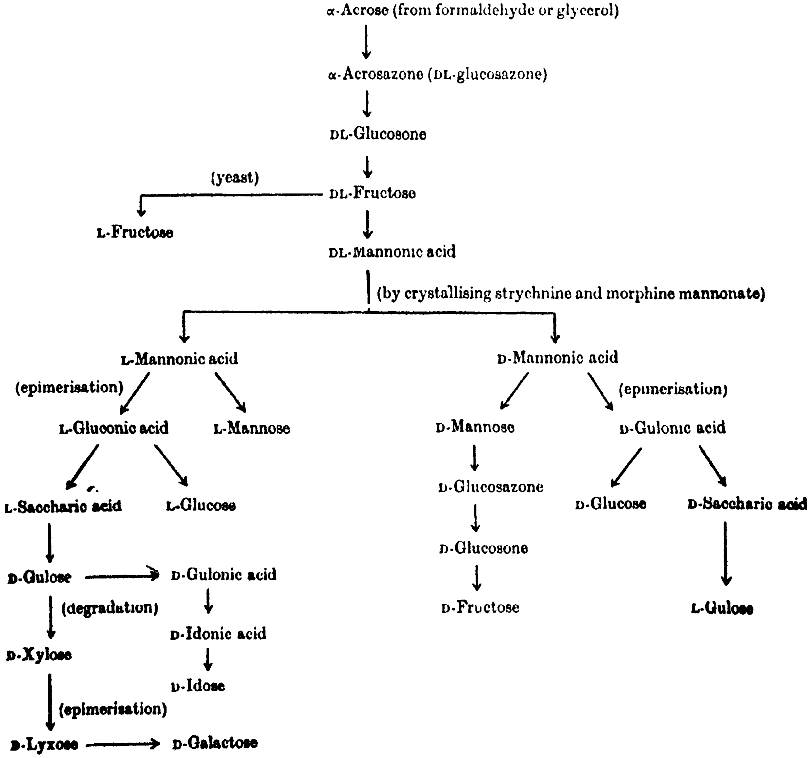
† *Ber.*, 1890, **23**, 370, 799, 2114.



$$\begin{array}{c}CH_2OH\\|\\CO\\|\\HOCH\\|\\HCOH\\|\\HCOH\\|\\CH_2OH\end{array}\xrightarrow{\text{Reduction}}\begin{array}{c}CH_2OH\\|\\HOCH\\|\\HOCH\\|\\HCOH\\|\\HCOH\\|\\CH_2OH\end{array}\xrightarrow{HNO_3}\begin{array}{c}COOH\\|\\HOCH\\|\\HOCH\\|\\HCOH\\|\\HCOH\\|\\CH_2OH\end{array}$$

D-Fructose D-Mannitol D-Mannonic acid

into its optical isomers by combination with strychnine and with morphine, and the transformations indicated below in tabular form were carried out by the general methods already outlined.



Production of Sugars from Acrose

CHAPTER II

THE SUGARS AS RING FORMS

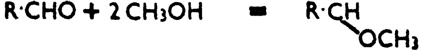
The reader will recall (page 5) that although in some important reactions a sugar such as glucose appears to behave as an aldehyde, in others, notably in its failure to react with Schiff's reagent under normal conditions, and even in such an obvious feature as smell, no aldehydic property is discernible. Although ring formulae were therefore proposed, notably by Tollens (1883) (page 5), to explain these apparent anomalies, many years were to elapse before such views became generally accepted. Attention at this time was mainly focussed on the work of Emil Fischer and it is unfortunate that Fischer himself did little to further the acceptance of ring formulae for the free sugars. However just as Priestley's discovery of oxygen contributed most to the downfall of the Phlogiston Theory so also an important discovery made by Fischer played a great part in paving the way for the adoption of cyclic sugar formulae. This was the synthesis of compounds called glucosides by the condensation of various sugars such as glucose, arabinose and galactose with alcohols such as methanol and ethanol.*

The Methylglucosides

By heating glucose† in a sealed tube with methanolic hydrogen chloride, a crystalline substance, methylglucoside [$C_6H_{11}O_5(OCH_3)$], m.p. 165°, $[\alpha]_D + 157°$, was isolated. Since it contained only one methoxyl residue and analysis showed the above formula to represent the compound, the equation for its formation must be written

$$C_6H_{11}O_5(OH) + HOCH_3 = C_6H_{11}O_5(OCH_3) + H_2O$$

Methylglucoside does not react with phenylhydrazine and is non-reducing, but on boiling with dilute mineral acids or by the action of maltase the above reaction is reversed. Since aldehydes normally combine with alcohols to form acetals, Fischer supposed that glucose



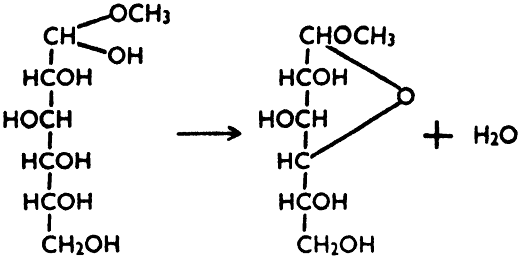
$$R \cdot CHO + 2CH_3OH = R \cdot CH\begin{matrix}\nearrow OCH_3 \\ \searrow OCH_3\end{matrix}$$

---

* Fischer, *Ber.*, 1893, **26**, 2400.

† For convenience the prefix D will be omitted in future unless there is a special reason for including it.

combined with one molecule of methanol to form a hemiacetal which, by the elimination of water between the new hydroxyl group on C_1



and that on C_4 gave the methylglucoside. The new compound, by analogy with the stable γ-lactones, was represented as containing a five-membered oxide ring. Fischer realised that a new " asymmetric carbon atom " had been introduced on C_1, and that theoretically therefore there should be two methylglucosides. In 1894 Alberda van Eckenstein* isolated a second isomer, β-methylglucoside, m.p. 104°, with a much lower optical rotation ($[\alpha]_D - 33°$); he called Fischer's product α-methylglucoside and pointed out the probability of a similar kind of relationship between the two isomeric glucose pentaacetates.† Although Fischer recognised that his discovery of methylglucoside meant the introduction of ring formulae and indicated how two simple sugars could be combined in such disaccharides as sucrose and lactose, he was careful to point out that it did not constitute confirmation of a ring structure, as suggested by Tollens, for the parent sugars.

The Isomeric Glucoses

Attention was next focussed on glucose itself owing to the discovery by C. Tanret‡ of a second form of glucose. The possibility that such a compound could exist had always been present ever since Dubrunfaut§ had discovered the " birotation " of glucose in 1846. When ordinary crystalline glucose (dextrose) is dissolved in water the initial specific rotation, $[\alpha]_D + 111°$, gradually falls to about half this value ($+52{\cdot}5°$). The halving of the rotation was not found to be general for all the sugars, galactose, maltose, and lactose being exceptions, so the name was changed to multirotation and finally, by Lowry,* to mutarotation. Crystallising ordinary α-glucose at temperatures above 98°, Tanret isolated an isomer, $[\alpha]_D + 19°$, originally called by him γ-glucose, since β-glucose was the name given at that time to the product, which was really a mixture, having $[\alpha]_D + 52{\cdot}5°$. The obvious similarity between the orders of the specific rotations of the α- and γ-methylglucosides was commented on by L. J. Simon† and the direct relationship between them was proved in the following neat experiment by one of Fischer's pupils, E. F. Armstrong.‡ α-Methylglucoside is hydrolysed in the presence of maltase, to glucose, whereas β-methylglucoside is similarly hydrolysed in the presence of emulsin, the enzymes in question being specific for α- and β-glucosides respectively. The experiment depended on the fact that although the mutarotation of glucose in pure water takes place in a matter of hours, it is virtually instantaneous in the presence of a trace of alkali.§ Armstrong took a solution of β-methylglucoside and treated it with emulsin, observing the optical rotation at intervals before and after the addition of a drop of ammonia solution. He found that after the hydrolysis had proceeded for a time, the rotation rose on the addition of ammonia. This indicated β-glucose (originally named γ-glucose by Tanret) to be present in the solution since the accelerated mutarotation was in an upward direction. When, on the other hand, a trace of ammonia was added to a solution of α-methylglucoside to which maltase had been added and hydrolysis allowed to proceed, the rotation fell, indicating that the free glucose present was the α-form. In a typical experiment a 10% solution of α-methylglucoside with maltase, after standing for 2 hours, had $\alpha_D = +14°36'$ falling to $+13°40'$ on the addition of ammonia. This experiment therefore strongly supported Simon's view that α-glucose was related to α-methylglucoside and β-glucose to β-methylglucoside.

---

* *Rec. trav. chim.*, 1894, **13**, 183.

† Franchimont, *Rec. trav. chim.*, 1893, **12**, 310.

‡ *Compt. rend.*, 1895, **120**, 1060.

§ *Compt. rend.*, 1846, **23**, 38.

Leaving aside for the moment the precise ring structures concerned, and adopting the convention that where the methoxyl group in the D-series is on the right hand side we are dealing with the α-form, we have to consider the arrangements below. First of all it should be emphasised once more that the conversion of C_1 to an asymmetric centre doubles the number of isomeric sugars in any one

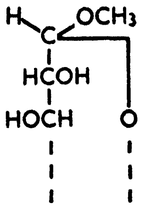
---

* *J.C.S.*, 1899, **75**, 211.

† *Compt. rend.*, 1901, **132**, 487.

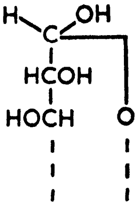
‡ *J.C.S.*, 1903, 1305.

§ Lowry, *J.C.S.*, 1903, 1314.



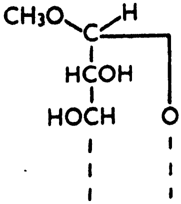
α-Methyl-D-glucoside

$[\alpha]_D + 157°$



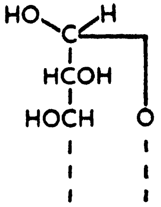
α-D-Glucose

$[\alpha]_D + 111°$



β-Methyl-D-glucoside

$[\alpha]_D - 33°$



β-D-Glucose

$[\alpha]_D + 19°$

group, thus there are now thirty-two hexoses and sixteen pentoses. It is important to remember that α- and β-D-glucose are *not* enantiomorphs, but are known as *anomers*; except that the groups on C_1 readily undergo interconversion, they are as different as say α-D-glucose and α-D-galactose, each pair differing by the stereochemical arrangement around one carbon atom (C_4 in the latter example).

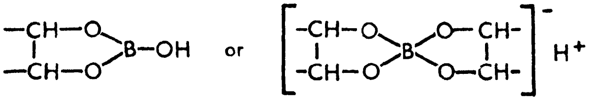
To avoid confusion in the matter of the new isomers or *anomers* a convention was proposed by Hudson* which is generally accepted. This states that for pairs of α- and β-isomers in the D-series, the one with the highest rotation in the positive sense shall be called the α-form, whereas the isomer of the lowest rotation is designated β. The reverse holds good in the L-series, the enantiomorph of α-D-glucose being α-L-glucose with a lower specific rotation (calc. −111°) than β-L-glucose (calc. −19°).

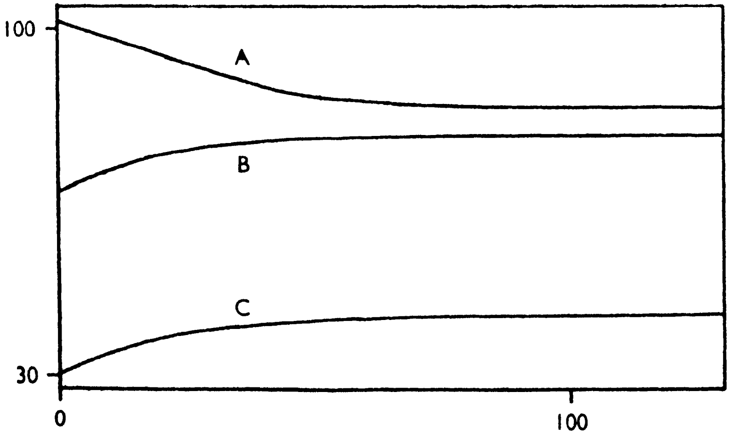
The next point to consider is whether what may seem to the reader to be somewhat arbitrary decisions as to the positions of the groups on C_1 have any relation to the known facts. The most comprehensive indication that they are in fact correct has been obtained from the results of X-ray analysis. The chemical study of cellulose for example has revealed that the glucose units which are its building stones are united by β-links. Thus cellulose and its derivatives have low specific rotations (cf. β-glucose), and its degradation product cellobiose is hydrolysed by emulsin which is specific for β-linkages. X-ray analysis shows that only by adopting the arrangement of glucose units joined on the pattern indicated above for β-methylglucoside can the calculations made from the X-ray photographs be interpreted. More direct still has been the application of X-rays to glycosides and free sugars by E. G. Cox and his co-workers.†

* *J. Amer. Chem. Soc.*, 1909, **31**, 66.

† *J.C.S.*, 1935, 1495.

Certain chemical evidence has also been adduced to confirm the above arrangements and one direct method introduced by J. Böeseken* is worthy of mention. As a result of a study of glycols, Böeseken found that *cis*-α-glycols enhanced the conductivity of aqueous boric acid. He attributed this to the formation of complex ions, the ionisation of the feebly dissociated boric acid being enhanced thereby. Accordingly this method was applied to the two forms of D-glucose with the following result. When α-D-glucose was dissolved in a solution of boric acid the specific conductivity increased markedly and then gradually fell at a rate comparable with the mutarotation. On the other hand the conductivity of β-D-glucose in boric acid was very much lower than that of the α-form and the specific conductivity of the solution rose during mutarotation to about the same equilibrium value.



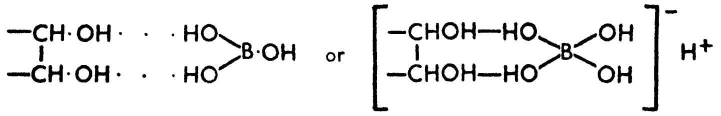


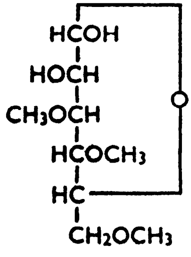
Ordinate: Conductivity of Sugars in M/2 Boric Acid Solution, $k \times 10^6$
Abscissa: Time in Minutes
A: α-D-Glucose **B:** β-D-Glucose **C:** 3,4,6-Trimethyl α-D-mannose

It is not quite certain that the structures of the boric acid complexes suggested by Böeseken are correct, for it seems unreasonable to suppose that definite anhydrides should be formed in aqueous solution, and the formation of " hydrogen bonds " due to resonance between the sugar hydroxyls and boric acid, for example with the displacement of charges resulting in the increase in electropositive character of the central boron atom, is perhaps a more probable explanation.* Nevertheless there is no doubt that, whatever the mechanism involved, *cis*-glycols, with adjacent hydroxyl groups enhance the electrical conductivity of boric acid and the presumption is, therefore, that α-D-glucose has a pair of such *cis*-hydroxyl groups and on mutarotation the effect disappears, whereas with β-D-glucose a *cis* α-glycol appears to be formed during mutarotation. From this result the point was made by Böeseken that in α-D-glucose the hydroxyl groups on C_1 and C_2 are *cis* whereas in the β-form they are *trans*, as given in the formulations on page 28. To determine if the other free hydroxyl groups in glucose played any part in this effect 3,4,6-trimethyl α-D-mannose was examined* using Böeseken's method. Since mannose and glucose are epimeric, an effect like that observed with β-D-glucose should have been observed since the hydroxyl groups are *trans* in the crystalline starting material. This was in fact the case; during mutarotation in boric acid solution the specific conductivity rose, thus justifying Böeseken's conclusions.

---

* *Ber.*, 1913, **46**, 2612.





3,4,6-Trimethyl α-D-mannose

Apart from Böeseken's investigations Rüber† has shown that in general a slightly higher molecular refraction is shown by *trans* compounds than by the corresponding *cis* derivatives, and it is found that β-D-glucose, β-D-galactose and α-L-arabinose have molecular refractions higher by about 7% than their isomers, a fact which agrees with the *trans* arrangement in these cases.

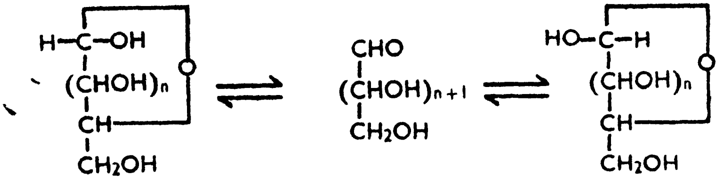
---

* Macpherson and Percival, *J.C.S.*, 1937, 1920.

† *Norske, Forh.*, 1931, **4**, 157.

Mutarotation

This phenomenon is explained as the reversible transformation of one isomeric form into the other, most probably through the intermediate production of the aldehydic form.* At equilibrium the proportion of α- and β-forms is not necessarily equal, and it varies for different sugars. Naturally the sign of rotation of the sugar in solution at equilibrium depends on the relative proportions of the two forms present but it is clear that a system of classification based on actual determinations of the sign of rotation at equilibrium is of little use (see page 13). Lest the reader should lose sight of the possibility, it should be emphasised that for all sugars capable of existing in ring forms, that is from the tetroses upwards, there can exist not only α- and β-forms of the sugar but also of its derivatives such as the methylated sugars, the acetates, the acetohalogenoses (that is acetates with a halogen atom replacing the reducing hydroxyl group) and so on, although it is not always practicable to isolate both the isomers in every case. The situation is complicated further since several ring systems are possible, and for each ring system two forms, α and β, automatically exist.



α-Form β-Form

The Evaluation of the Ring Structures

We have left aside the question of the actual ring forms both in the sugars and the methylglycosides,† but this issue can be evaded no longer. It has been made clear that chemists were obliged to accept a ring structure for the methylglycosides and Fischer, presumably influenced by the early Tollens formula, represented these compounds by 1,4- or γ-oxide ring structures like the stable γ-lactones. This view was not seriously challenged until 1925 when a mass of evidence was accumulated by Sir W. N. Haworth* which successfully resolved all the difficulties.

---

* Lowry, *Optical Rotatory Power*, 1935, p. 273. Longmans, London.

† The general term for analogues of methylglucoside when a sugar other than glucose is concerned is glycoside.

It is necessary to consider at this point the discovery by Emil Fischer† of what appeared to be a third methylglucoside which he named "γ"-methylglucoside. This substance, formed by reacting on glucose with dilute methanolic hydrogen chloride at room temperature, was not crystalline but distilled fairly readily in a high vacuum; other "γ"-methylglycosides were obtained from galactose, mannose, and pentoses by a similar process. As a class these compounds were characterised by their special property of undergoing very ready hydrolysis with exceedingly dilute mineral acids, to give the parent sugar and methanol. It was clear at once that a different ring system must be present in these new compounds, although it should be added that they subsequently proved to be mixtures since pure α- and β-forms in several cases were prepared much later by Haworth (see page 58). The alternatives to the generally accepted 1,4-oxide ring for the normal glycosides were 1,2-, 1,3-, 1,5- or 1,6- oxide rings. Stereochemically owing to the strain involved, the first two were unlikely possibilities and the 1,5-oxide ring was generally preferred for the "γ"-glycosides.

The attack on the general problem of ring structure fell into two parts, oxidative degradations of methylated sugars and a study of the lactones of the sugar acids.

It is necessary at this point to digress for a moment to consider why methylated sugars played, and are still playing, a most important role in carbohydrate chemistry. Before any structural investigations can be made into compounds like the sugars which contain many hydroxyl groups as well as reactive potential aldehydic or ketonic groups and consequently possibilities of ring shift on every hand, it is obviously necessary to protect the hydroxyl groups in some way, and by a group that is not too large. This was recognised at the beginning of the century by Thomas Purdie‡ who, with the

---

* *The Constitution of Sugars*, 1928, Arnold, London.

† *Ber.*, 1914, **47**, 1980.

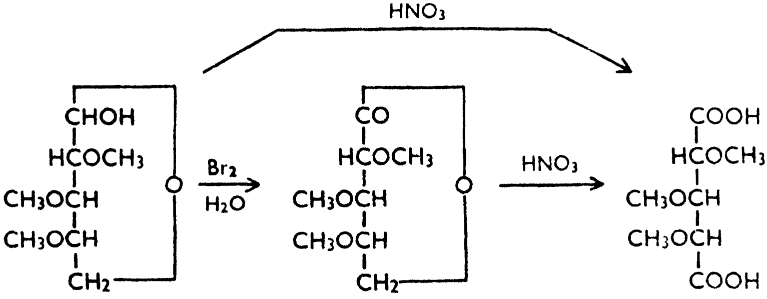
‡ Purdie and Irvine, *J.C.S.*, 1903, 1021.

St. Andrews chemists, notably Sir J. C. Irvine, initiated, in the course of a vigorous attack on the structure of the disaccharides and polysaccharides, the method of methylation with methyl iodide and silver oxide, and prepared a number of crystalline methylated sugars.

Haworth, also working at St. Andrews, developed a more efficient and rapid method of methylation with sodium hydroxide and methyl sulphate,* thus removing the methylated sugars from the position of chemical curiosities. It was then possible to examine critically the oxidation products of methylated sugars in which ring changes had been prevented by the stabilisation of the hydroxyl groups by substitution. Some typical examples are considered below.

The Methylarabinosides and Methylxylosides

When α- and β-methyl-L-arabinosides are methylated and hydrolysed, a trimethyl arabinose is obtained. Oxidation with nitric acid† gave L-*arabo*trimethoxyglutaric acid, evidence that in the trimethyl arabinose, the ring joined C_1 and C_5. When crystalline trimethyl arabinose was oxidised with bromine water an acid was obtained which on heating gave rise to a lactone. The properties of this lactone gave a clue to its structure; on standing in aqueous solution it underwent rapid hydrolysis to the free acid. This is characteristic of δ-lactones,‡ but is quite distinct from the behaviour of the more



Trimethyl-L-arabinose δ-Trimethyl L-arabonolactone L-*Arabo*trimethoxyglutaric acid familiar γ-lactones, which are only slowly hydrolysed to the free acids. The rate of hydrolysis may be measured by following the optical rotation, as it approaches zero on conversion to the acid, or by measurements of electrical conductivity, and a large number of lactones both unsubstituted and methylated, were examined by W. N. Haworth and his School by these methods.* Oxidation of the above trimethyl arabonolactone with nitric acid† gave the optically active L-*arabo*trimethoxyglutaric acid, which confirmed the conclusion that it was a δ- or 1,5-lactone and proved the trimethyl arabinose to be the 2,3,4-trimethyl derivative.

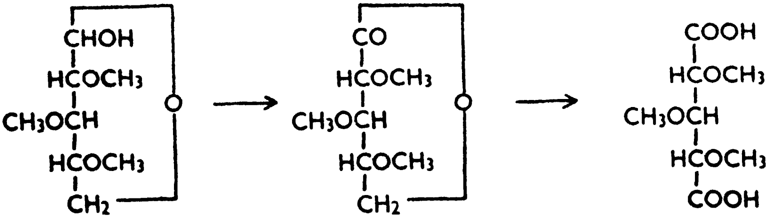
---

* *J.C.S.*, 1915, 8.

† Hirst and Robertson, *J.C.S.*, 1925, 358.

‡ Nef, *Annalen*, 1914, **403**, 204.

When the crystalline trimethyl xylose prepared by the methylation of β-methylxyloside was examined, it too was found to give on oxidation with bromine water a readily hydrolysable lactone, which was proved to be 2,3,4-trimethyl δ-xylonolactone, oxidation with nitric acid† yielding i-*xylo*trimethoxyglutaric acid. This acid, which had been obtained previously by the oxidation of the free trimethyl sugar‡ is a *meso* form and its isolation, in the form of its crystalline amide, proves that the lactone and the original trimethyl xylose, and hence the methyl-D-xyloside, have the same kind of six-membered ring structure, as the methyl-L-arabinosides.



2,3,4-Trimethyl D-xylose

δ-Trimethyl D-xylonolactone

i-*Xylo*trimethoxyglutaric acid

The Methylglucosides and Methylgalactosides

When the α- and β-methylglucosides are methylated and hydrolysed, a crystalline tetramethyl glucose§ is produced, m.p. 96°

---

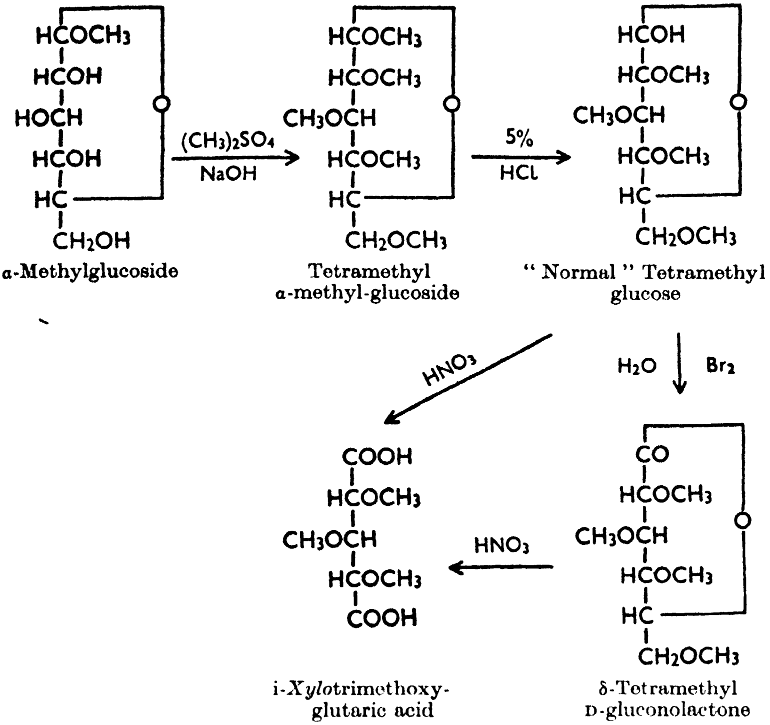
* Drew, Goodyear and Haworth, *J.C.S.*, 1927, 1237.

† Haworth and D. I. Jones, *J.C.S.*, 1927, 2349.

‡ Hirst and Purves, *J.C.S.*, 1923, 1352.

§ Purdie and Irvine, *J.C.S.*, 1903, 1021; 1904, 1049.

$[\alpha]_D + 84°$, which is recognisable as similar to α-D-glucose and is quite normal in its properties.



α-Methylglucoside

Tetramethyl α-methyl-glucoside

" Normal " Tetramethyl glucose

i-*Xylo*trimethoxy-glutaric acid

δ-Tetramethyl D-gluconolactone

Oxidation with bromine water gave a tetramethyl gluconolactone easily hydrolysed by water and belonging therefore to the δ-series, and which gave,* as did the tetramethyl glucose† itself, on oxidation with nitric acid the same i-*xylo*trimethoxyglutaric acid as that obtained from trimethyl xylose.

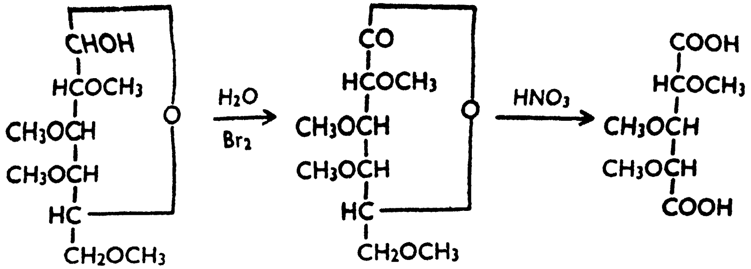
In a similar way the crystalline methylgalactosides may be converted into a " normal " tetramethyl galactose. This substance on oxidation gave a lactone with the typical properties of a δ-lactone and further oxidation with nitric acid gave the same L-*arabo*trimethoxyglutaric acid which we have previously described as obtained from the methylarabinosides.‡

---

* Haworth, Hirst and Miller, *J.C.S.*, 1927, 2436.

† Hirst, *J.C.S.*, 1926, 350.

‡ Haworth, Hirst and D. I. Jones, *J.C.S.*, 1927, 2428.



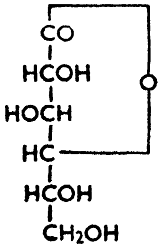
Tetramethyl galactose

δ-Tetramethyl D-galactonolactone

L-*Arabo*trimethoxyglutaric acid

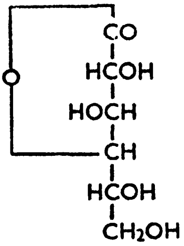
This series of reactions both for the glucose and galactose derivatives leaves no room for doubt that the methyl-glucosides and -galactosides have 1,5-oxide ring structures and not 1,4- or (γ-oxide) rings as Fischer had assumed.

An interesting point of great consequence may be noted about the tetramethyl δ-galactonolactone mentioned above. It has a positive rotation, unlike the laevorotatory γ-D-galactonolactone or the 2,3,5,6-tetramethyl D-galactonolactone. This is an illustration of Hudson's lactone rule.* By examining the properties of a large number of crystalline sugar lactones Hudson came to the conclusion that if in the projection formula the lactone ring was on the right hand side, then the rotation of the acid was increased in a positive sense on lactonisation, whereas if it were written on the left hand side the reverse was true. Two examples of this are given below. The cause of this considerable change in rotation on the lactonisation of an acid is connected with the torsional effect involved in bringing the lactone ring into the same plane as that of the other carbon



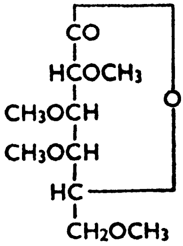
γ-D-Gluconolactone

[α]D +68°



γ-D-Galactonolactone

[α]D − 77°



δ-Tetramethyl D-galactonolactone

[α]D +145°

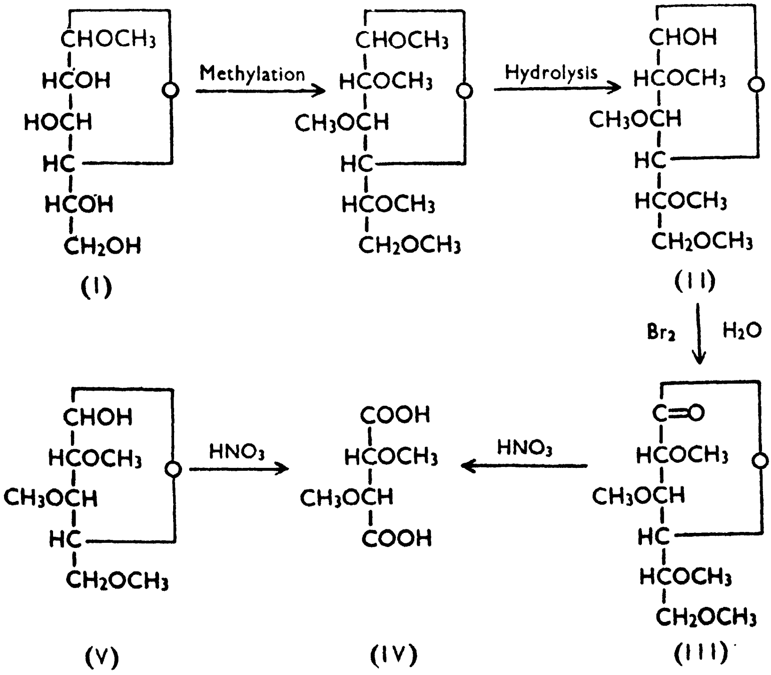
---

* *J. Amer. Chem. Soc.*, **1910, 32, 338.**

atoms. Although at first sight the rule appears rather empirical it has proved very useful, for example in the methylated galactonolactone discussed above, since the hydroxyl groups on C_4 and C_5 are on opposite sides of the projection formula, inspection of the specific rotation decides whether the lactone in question is a δ- or γ-lactone. H. D. K. Drew and W. N. Haworth* considered the application of Hudson's lactone rule to the sugars themselves, in a very important paper which appeared in 1926. It was pointed out that grave anomalies existed when the sugars were considered as containing 5-atom rings, which vanished when the 6-atom ring structure was substituted. Thus on the basis of the five-atom ring structure for D-galactose, the equilibrium rotation of the sugar would be expected to be strongly negative like the γ-lactone, whereas in fact it is strongly positive.

The "γ"-Methylglycosides of Fischer

Methylation and hydrolysis of the liquid "γ"-methylglucoside (I) obtained by the action of cold methanolic hydrogen chloride on



---

* *J.C.S.*, 1926, 2303.

glucose by Irvine and his co-workers,* gave a liquid tetramethyl glucose (II). When examined by the methods just outlined, this substance was shown to give on oxidation with bromine water a crystalline tetramethyl gluconolactone (III)† which was a typical γ-lactone since it was only hydrolysed slowly by water, and in its general properties and those of its derivatives was quite different from the 2,3,4,6-tetramethyl gluconolactone already described and obtained from normal tetramethyl glucose. Further proof was afforded since on oxidation with nitric acid a mixture of L(+)-dimethoxysuccinic acid (IV) and oxalic acid was obtained.‡ When "γ"-methylxyloside was methylated and hydrolysed, the trimethyl xylose (V) produced was proved to be 2,3,5-trimethyl xylose§ since oxidation gave the same derivative of tartaric acid, and the lactone obtained on oxidation with bromine water was a typical γ-lactone as judged by its slow hydrolysis to acid.

The Methylfructosides

Although we have not previously mentioned the fact, the ketose, fructose, reacts with methanolic hydrogen chloride in a similar way to the aldoses. Thus in the cold a non-reducing syrup, "γ"-methylfructoside which is easily hydrolysed by very dilute acid, is obtained.

It is not quite such a straightforward task to determine the ring structure of ketose derivatives because direct oxidation to an acid and studies of the resulting lactone cannot be carried out. The following technique demonstrated‖ that the "γ"-methylfructoside like the corresponding glucose derivative contains a five-membered oxide ring system.

Methylation and hydrolysis affords a syrupy "γ"-tetramethyl fructose, a product which is obtained more readily, however, from methylated cane sugar, with a positive rotation as distinct from the strongly laevorotatory fructose. This tetramethyl γ-fructose on oxidation with nitric acid was converted into a trimethyl 2-keto-gluconic acid, which on esterification and glycoside formation with methanolic hydrogen chloride followed by treatment with ammonia, gave a very useful crystalline reference compound, 2,3,4,6-tetramethyl 2-ketogluconamide, for this series which will be referred to later (page 93). The free trimethyl 2-keto-gluconic acid on oxidation with barium permanganate and sulphuric acid was converted into crystalline 2,3,5-trimethyl D-arabonolactone, the enantiomorph of the lactone prepared from "γ"-methyl-L-arabinoside. Further oxidation gave D(–)-dimethoxysuccinic acid.

---

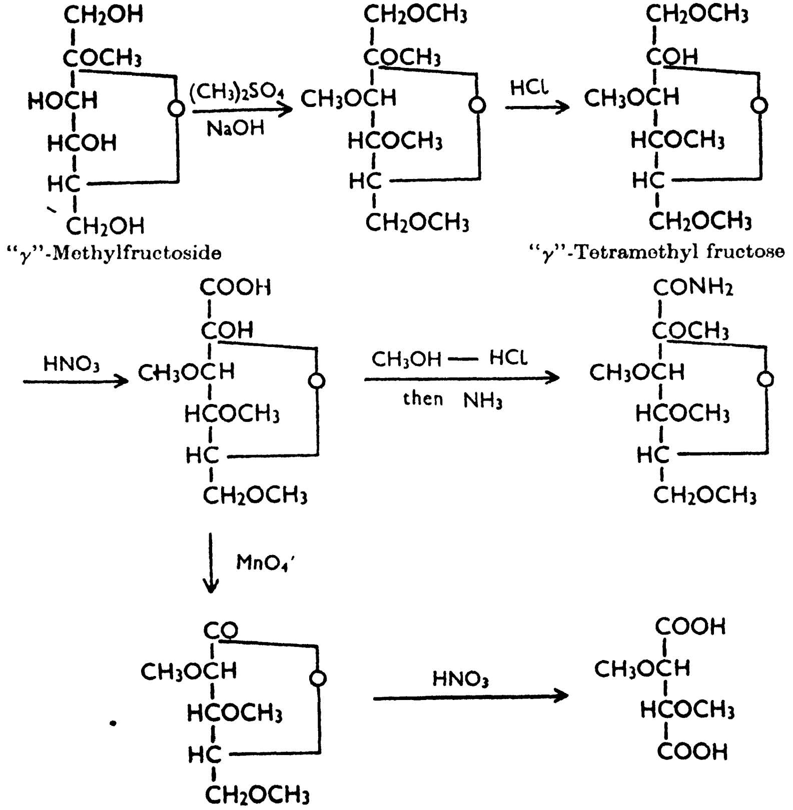
* Irvine, Fyfe and Hogg, *J.C.S.*, 1915, 524.

† Drew, Goodyear and Haworth, *J.C.S.*, 1927, 1237; Charlton, Haworth and Peat, *J.C.S.*, 1926, 100.

‡ Haworth, Hirst and Miller, *J.C.S.*, 1927, 2436, Ref. 28.

§ Haworth and Westgarth, *J.C.S.*, 1926, 880.

‖ Avery, Haworth and Hirst, *J.C.S.*, 1927, 2308.

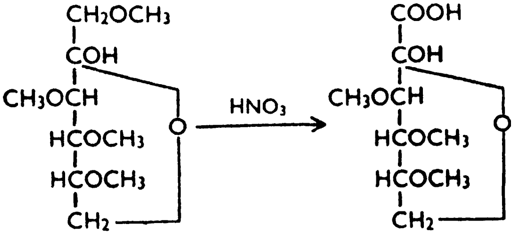


2,3,5-Trimethyl D-arabonolactone

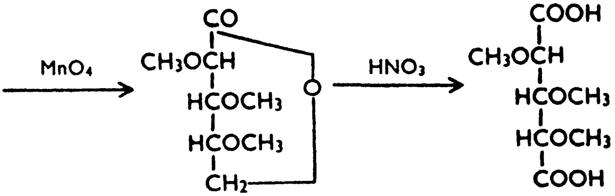
Normal methylfructosides are not prepared as simply as the corresponding glucose derivatives since "γ"-methylfructosides are formed at the same time. Nevertheless by a somewhat circuitous route crystalline β-methylfructoside, $[\alpha]_D - 172°$, has been prepared and from it a crystalline tetramethyl fructose* was obtained, which, if its specific rotation $[\alpha]_D - 123°$ is any guide must be considered as derived from ordinary fructose ($[\alpha]_D$ − 130°). Oxidation* followed a similar course to that described above but the lactone isolated on this occasion was δ-trimethyl D-arabonolactone which on further oxidation gave D-*arabo*trimethoxyglutaric acid. This brings out clearly the fact that the strongly laevorotatory tetramethyl fructose is 1,3,4,5-tetramethyl fructose with a six-membered oxide ring.

---

* Purdie and Paul, *J.C.S.*, 1907, **91**, 289; (Miss) E. S. Steel, *J.C.S.*, 1918, 257.



1,3,4,5-Tetramethyl D-fructose



δ-Trimethyl D-arabonolactone D-*Arabo*trimethoxy-glutaric acid

Glycosides both normal and labile ("γ") of all the important sugars have been examined by the above methods, and in each case it has been found that the normal glycosides, prepared using hot methanolic hydrogen chloride and requiring fairly strong mineral acid (say 3-5% HCl) for their hydrolysis have 1,5-oxide or amylene-oxide rings, whereas the easily hydrolysed "γ"-glycosides, prepared in the cold, invariably have 1,4-oxide rings. The reader will observe it was pure coincidence that Fischer called these latter compounds γ-glucosides to distinguish them from the crystalline α- and β-methylglucosides, and the fact that they were eventually shown to contain a γ-oxide ring was fortuitous.

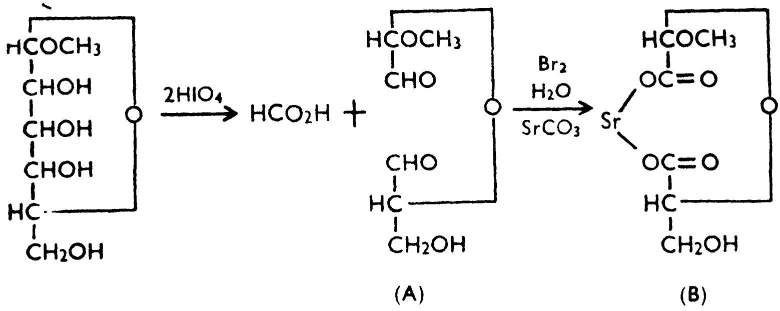
The Use of Periodate Oxidation in Structural Studies

Oxidation experiments with periodic acid carried out by Jackson and Hudson† have given striking confirmation that the above cyclic structures assigned to the methylglycosides are correct. The use of this reagent to oxidise α-glycols has already been mentioned in Chapter I in connexion with glucose, and, though in the example given the aldehydic structure of glucose was assumed, periodic acid is also capable of dealing with the α-glycol groups in cyclic structures in a similar way. The essential point to notice is that whereas the oxidative degradation experiments previously discussed have been concerned with the breaking of the oxide ring, it is the chain of carbon atoms which is broken by periodate whilst the oxide ring is left intact. All α-methyl-D-hexosides of the normal type give the same product, together with a molecule of formic acid, and two molecules of periodic acid are consumed. The oxidation product (A) is a dialdehyde

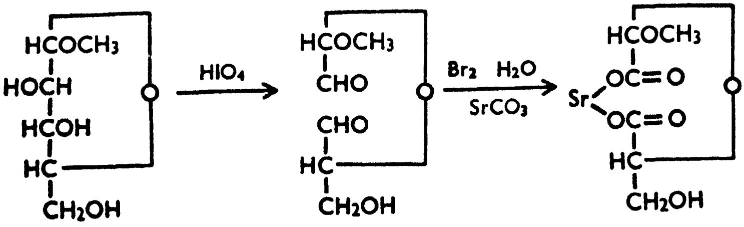
---

* Haworth and Hirst, *J.C.S.*, 1926, 1858; Haworth, Hirst and Learner, *J.C.S.*, 1927, 1040.

† *J. Amer. Chem. Soc.*, 1937, **59**, 994; Hudson, *J. Chem. Ed.*, 1941, **18**, 353.



and is a derivative of diglycollic aldehyde $OHC.CH_2OCH_2.CHO$. For convenience in characterisation it is oxidised with bromine water and converted into a crystalline strontium salt of the corresponding diglycollic acid (B), namely strontium D′-methoxy D-hydroxymethyl diglycollate. The asymmetric centres at C_1 and C_5 are not affected, hence the necessity to specify the configurations of each. It is important to notice that this result is quite independent of the arrangements on C_2, C_3 and C_4; in other words all " normal " α-methyl-D-hexosides would be expected to give the same product, and this as previously stated is indeed the case. A slightly different result is obtained where the β-isomers are used. Then the oxidation product has the configuration on C_1 reversed and the substance finally obtained is strontium L′-methoxy D-hydroxymethyl diglycollate. When the pure α-form of "γ"-methyl-D-arabinoside is oxidised the same product is obtained, with the difference that only one molecule of periodic acid is consumed and no formic acid is produced. When the normal methylpentosides, such as the pure α- and β-methyl-D-

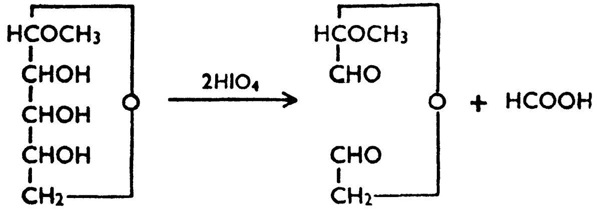


"γ"-Methyl-D-arabinoside (α-form)

(A)

(B)

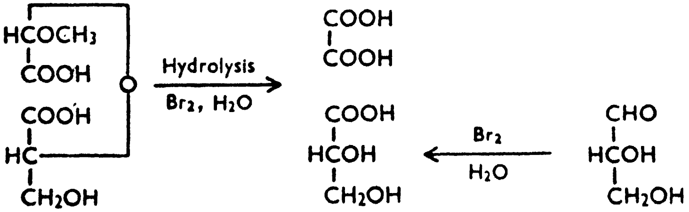
xylosides and D-arabinosides, are treated, monosubstituted diglycollaldehyde derivatives are obtained. These experiments give results therefore which fully confirm the findings of Haworth and his School.



α-Methyl-D-pentoside

D′-Methoxy diglycollaldehyde

One other significant fact emerges from the periodate oxidation of the methylhexosides. The oxidation product from the α-methylglucoside (B) [or from any other α-methyl-D-hexoside] is converted by hydrolysis in aqueous solution followed by oxidation with bromine water, into D(–)-glyceric acid and oxalic acid. D(–)-glyceric acid was obtained from D(+)-glycerose by Wohl, so that this result



D′-Methoxy D-hydroxymethyl diglycollic acid

D(–)-Glyceric acid

D(+)-Glycerose

proves that the configuration on the penultimate carbon atom of B and of α-methylglucoside is the same as in D-glycerose which, as shown in Chapter I, is the parent of the D-series of sugars. Fischer's proofs of the relative configurations of the sugars, for example glucose, depended on reactions involving the aldehydic form, and some workers had raised the question whether opening the ring from the stable cyclic form to give the open chain aldehyde had involved a Walden inversion on the carbon atom concerned, in this case on C_5. In such circumstances α-methylglucoside would really be α-methyl-L-idoside, but this confusing possibility is negatived by the experiments of Jackson and Hudson discussed above.

The Ring Structures of Free Sugars

The results of the methylation and oxidation experiments on the methylglycosides, whether the crystalline α- and β-forms or the syrupy γ-methylglycosides, do not of themselves allow us to decide the ring structures of the parent sugars. At the same time, however, there is strong circumstantial evidence from the experiments of E. F. Armstrong on the hydrolysis of the crystalline α- and β-methylglucosides with enzymes, that the α- and β-glucoses have the same ring structures as the corresponding glycosides (page 27), and to this must be added the similar specific rotations and indeed a mass of evidence based on calculations from the optical rotations of the sugars. Polarimetric observations by Isbell and Hudson* of the oxidation of aldoses with bromine water both in the presence of acetate buffer and of barium carbonate have shown that the easily hydrolysed δ-lactones are the first detectable oxidation products. Later† it was shown that crystalline δ-D-gluconolactone could be isolated after oxidising β-D-glucose with bromine water for half an hour in the presence of barium carbonate and extraction with dioxan. These results provide important additional evidence that in aqueous solutions of aldoses the six-membered ring forms predominate. Furthermore X-ray measurements on free sugars† have proved that *in the crystalline state* a six-membered ring is present.

Haworth's Nomenclature for the Ring Forms

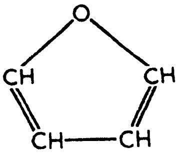
In order to systematise the somewhat haphazard way in which the ring forms of sugars and their derivatives were described, of which such examples as amylene oxide, γ-oxidic and similar terms have occurred in the preceding pages, Haworth‡ suggested that the five-atom ring sugars should be regarded as derived from furane and the six-atom ring compounds from pyrane. Thus a sugar containing a γ-oxide ring is called a *furanose* sugar, and if it is present as a glycoside it is described as a *furanoside.* One must emphasise, however, that *unsubstituted* furanose sugars have never been isolated although they may have a transient existence in solution. Although the 1,4-ring is stable and the 1,5-ring is relatively unstable in the lactones, the reverse is usually true for the sugars and glycosides. Furanose structures are therefore found either in substituted sugars such as 2,3,5,6-tetramethyl glucose where there is no free hydroxyl group on C_5, or, more commonly, in glycosides, disaccharides and polysaccharides.

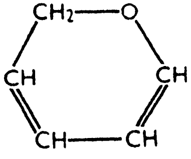
---

* *J. Res. Nat. Bur. Stand.*, 1932, **8**, 327, 615.

† Isbell and Pigman, *ibid.*, 1933, **10**, 337.

‡ Goodyear and Haworth, *J.C.S.*, 1927, 3136.

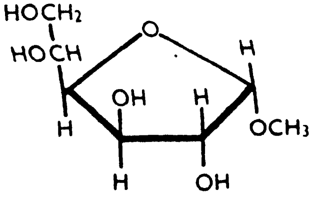

Furane

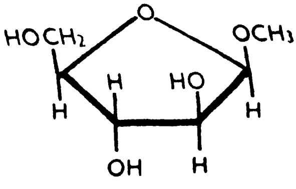

Pyrane

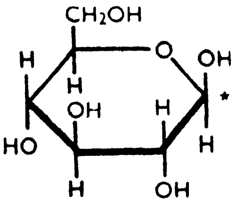
Similarly the expressions *pyranose* and *pyranoside* are applied to the sugar derivatives with six-membered rings.

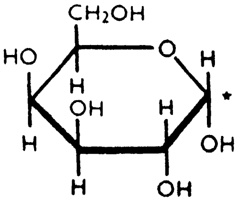
In this way one of the stereochemically pure forms of Fischer's original "γ"-methylglucoside now becomes α-methyl-D-glucofuranoside and the crystalline α-methylglucoside of Fischer, m.p. 165°, is named α-methyl-D-glucopyranoside.

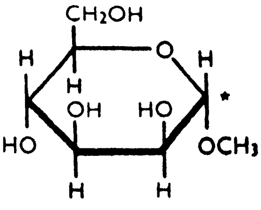
Pyranose and furanose rings are typified in the following formulae:

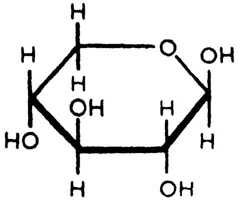

α-Methyl-D-glucofuranoside

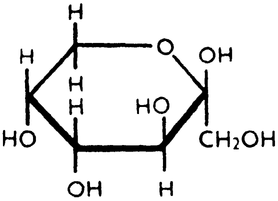

β-Methyl-D-arabofuranoside

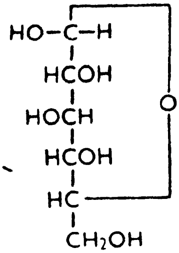

β-D-Glucopyranose

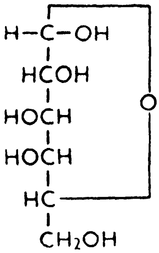

α-D-Galactopyranose

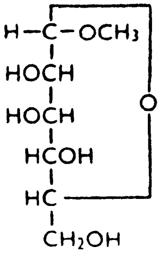

α-D-Methylmannopyranoside


β-D-Xylopyranose

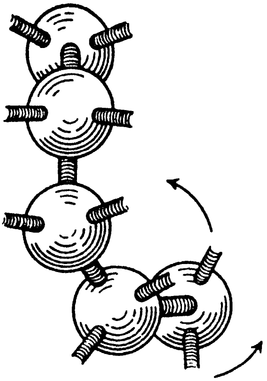

β-D-Fructopyranose

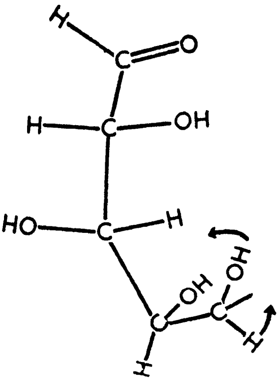

β-D-Glucopyranose


α-D-Galactopyranose


α-D-Methylmannopyranoside

the first three rows are of the type introduced by Haworth and are designed to show as precisely as possible the actual arrangement of the atoms and groups. In these formulae the ring planes are projecting from the plane of the paper with thickened edges outermost. The reader will notice, however, what would seem to be an obvious discrepancy between the two sets of formulae. Taking β-D-glucopyranose the sequence appears to be OH, H, OH, H, CH_2OH for the groups above the plane of the pyranose ring, whereas in the cyclised projection formula the sequence is OH, H, OH, H, H. The reason for the apparent anomaly is due to the torsional effect of ring closure. It must be remembered that to make a ring from the "straight chain" aldehydic form, the oxygen atom in the hydroxyl group on C_5 must be brought into the plane of the first five carbon atoms. The result of this is that the hydrogen atom attached to the same carbon atom now takes its place on the other side of the chain because the hydroxyl bond is rotated through more than a right angle. The reason for this apparent anomaly is therefore purely a mechanical one, but must always be considered when any ring formulae are constructed from the projection formulae. The best way to illustrate this is by the use of models, but in default of these, the student can take two pieces of copper wire, one piece say six inches long is bent in the form of an arc of a circle and a shorter piece, two inches long, bent in the middle roughly at





the tetrahedral angle (109·5°) is soldered, at the apex, about one inch from one end of the long strip. When one of the short ends is then joined to the end of the arc furthest away, it will be found that the other end of the short piece will have crossed over the plane containing the arc.

It should be pointed out also that the " model formulae " are idealistic in the sense that X-ray evidence shows that the pyranose ring is not quite flat, but takes up a strainless form; the furanose ring however is generally considered to be flat.

The Equilibrium between Ring Forms and Open Chain Forms

Although this chapter has been especially concerned with the development of the proofs of ring structure, and indeed the compounds we shall be dealing with later will be mainly cyclic, it is perhaps desirable to emphasise once more that for free sugars with no substituents to hinder inter-conversion there exists in solution equilibria between the forms. For example when fructose or galactose are methylated, mixtures of methylated furanosides and pyranosides are obtained. With galactose in solution for example a complex state of affairs (page 47) is said to exist.* According to Lowry† during the mutarotation of glucose, too the four ring forms and the aldehydic form are in equilibrium. The proportion of the aldehydic or ketonic form is very small indeed, although more marked in solutions of some sugars than others; fructose and sorbose, for example, show traces of the acyclic forms when their absorption spectra are examined, and 5-methyl glucose gives a positive test with Schiff's

---

* Schlubach and Prochownick, *Ber.*, 1929, **62**, 1502.

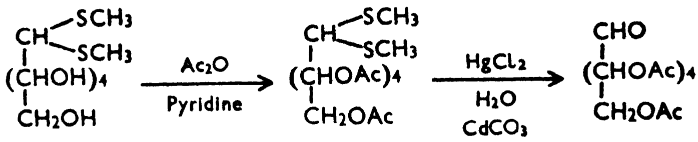
† Lowry, *Optical Rotatory Power*, 1935, p. 273, Longmans, London.

$$\begin{tikzcd}
\alpha\text{-D-Galactopyranose} \arrow[dr, shift left] & & \beta\text{-D-Galactopyranose} \arrow[dl, shift left] \\
& \text{Aldehydic form} \arrow[ul, shift left] \arrow[ur, shift left] \arrow[dl, shift left] \arrow[dr, shift left] & \\
\alpha\text{-D-Galactofuranose} \arrow[ur, shift left] & & \beta\text{-D-Galactofuranose} \arrow[ul, shift left]
\end{tikzcd}$$

reagent.* In most cases the aldehydic form cannot be detected by any means, although solutions of glucose and arabinose in 50% sulphuric acid show an absorption band in the ultra violet characteristic of a carbonyl group which gradually increases in intensity.†

Aldehydo-Sugars and Septanoses

It is perhaps appropriate to mention here the outstanding work of M. L. Wolfrom‡ on the isolation of *aldehydo*-sugars, because it serves to emphasise that although the reactions of a sugar in solution may arise directly through the aldehydic form, in its stable state it is cyclic. *Aldehydo*-sugars can only be isolated if all the hydroxyl groups are protected, for example, by acetyl groups. To prepare crystalline 2,3,4,5,6-pentaacetyl *aldehydo*-glucose, Wolfrom acetylated glucose dimethyl mercaptal, and decomposed the product with mercuric chloride in the presence of cadmium carbonate. The acetate obtained, unlike either of the cyclic glucose pentaacetates had a very low optical rotation, in harmony with its open chain structure, reacted immediately with semicarbazide, restored the colour to Schiff's reagent and reduced Fehling's solution. Similar substituted *aldehydo*-sugars have been prepared from galactose, xylose, arabinose, etc., and their general behaviour is such that confusion with ordinary sugars is impossible. Thus they readily form hydrates, alcoholates (cf. chloral), dimethyl acetals and bisulphite compounds,



$$CH(SCH_3)_2\text{–}(CHOH)_4\text{–}CH_2OH \xrightarrow[\text{Pyridine}]{Ac_2O} CH(SCH_3)_2\text{–}(CHOAc)_4\text{–}CH_2OAc \xrightarrow[H_2O,\ CdCO_3]{HgCl_2} CHO\text{–}(CHOAc)_4\text{–}CH_2OAc$$

---

* v. Vargha, *Ber.*, 1936, **69**, 2098.

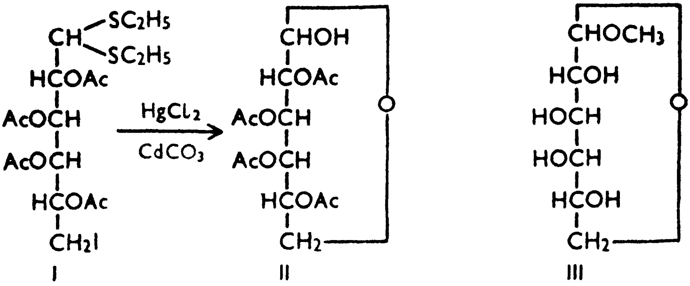
† Pacsu and Hiller, *J. Amer. Chem. Soc.*, 1948, **70**, 523.

‡ *J. Amer. Chem. Soc.*, 1929, **51**, 2188; 1930, **52**, 2464 et seq.

in short, behave as typical aldehydes. This work is therefore an important, if indirect, confirmation of the theory of the ring formulations discussed above.

Aldehydo-sugars must therefore be regarded as abnormal and another abnormal state of affairs may be mentioned, namely the derivatives of seven membered ring sugars or septanoses discovered by Micheel and Suckfüll.*

By treating tetraacetyl diethyl mercaptogalactose 6-iodide (I) with mercuric chloride and cadmium carbonate in the cold 2,3,4,5-tetraacetyl D-galactoseptanose (II) was isolated. The methylseptanoside (III) derived from this substance was found to undergo hydrolysis with very dilute hydrochloric acid (N/100) at a rate comparable with that of furanosides. Septanose derivatives have not however been isolated except by special methods such as described above, although it is not impossible that methylseptanosides could exist in equilibrium with the furanosides when a sugar such as galactose is treated with cold methanolic hydrogen chloride.



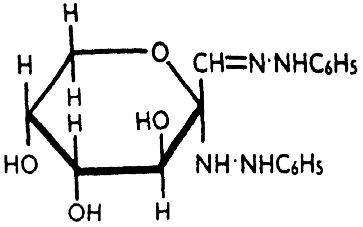
Other Cyclic Sugar Derivatives

Although emphasis has been laid upon the cyclic structures of the free sugars and the methylglycosides in this chapter, it should be pointed out that other sugar derivatives such as the sugar acetates (see page 53), acetone compounds (page 54), acetohalogenoses (page 60) and many other derivatives are cyclic. Sugar hydrazones and osazones can exist in cyclic as well as acyclic forms,† and since glucose phenylosazone on methylation gives rise to a trimethyl N-methyl phenylglucosazone, and the O-methyl groups have been shown to occupy positions 3, 4 and 5, this familiar compound has been formulated as phenyl D-fructopyranosazone.*

---

* *Annalen*, 1933, **507**, 138.

† See Percival, *Advances in Carbohydrate Chemistry*, 1948, **3**, 23.



For the sake of clarity, in future cyclic sugar formulae the hydrogen atoms directly attached to the rings will be omitted (see page 51).

---

* E. E. Percival and E. G. V. Percival, *J.C.S.*, 1935, 1398.

CHAPTER III

CHARACTERISTIC REACTIONS OF SUGARS INVOLVING HYDROXYL GROUPS

HAVING established that a hexose is, in its most stable form at any rate, a cyclic compound with five hydroxyl groups as addenda, we can consider some of the more important reactions which depend on the specific alcoholic properties of these groups.

Etherification

Methylation

The importance of methylation in the protection of the hydroxyl groups in sugars has been mentioned already. This object is usually achieved by the use of methyl sulphate and sodium hydroxide often followed by the use of Purdie's reagents, silver oxide and methyl iodide. These latter substances cannot be used directly to methylate a free reducing sugar because of the oxidising action of the silver oxide, and even when methylating a reducing sugar with methyl sulphate and alkali it is usual to adopt special precautions to prevent Lobry de Bruyn transformations (page 16), and more extensive degradation due to the oxidation of enolic forms. It is in fact always preferable, when possible, to use the methylglycosides as starting materials, although this is not always convenient, particularly with disaccharides. Other methods which have been used in suitable cases are treatment in liquid ammonia solution with potassium or sodium,* and methylation of the resulting alkali derivative with methyl iodide; where one hydroxyl group only is available for methylation and the substance is soluble in ether, for example diacetone glucose, the compound can be treated with sodium in ether to give an alcoholate followed by interaction with methyl iodide after removal of the solvent.† The method of R. C. Menzies‡ has, especially in the hands of Hirst and his co-workers, proved useful in special instances. This involves a replacement of the hydrogen atoms in the hydroxyl groups by thallium, applied either as thallous ethylate ($TlOC_2H_5$) or as the hydroxide, followed by treatment with methyl iodide.

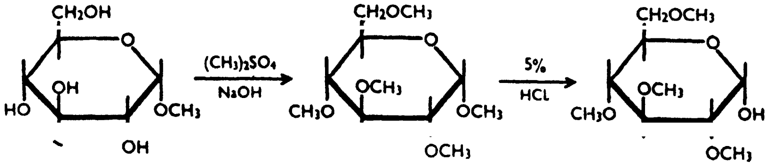
---

* Muskat, *J. Amer. Chem. Soc.*, 1934, **56**, 693, 2449. Hendricks and Rundle, *ibid.*, 1938, **60**, 2563.

† Freudenberg and Smeykal, *Ber.*, 1926, **59**, 100.

‡ *J.C.S.*, 1926, 937; 1947, 1378.

Fully methylated sugars for example the tetramethyl methylglucopyranosides are mobile oils, which are readily distilled in a high vacuum, and from which the glycosidic methoxyl residue is easily removed on hydrolysis with mineral acids to give the fully methylated sugar, in this particular case tetramethyl glucopyranose. The methoxyl residues, apart of course from that on C_1, the so-called reducing group, are stable to mineral acids except to very concentrated hydrochloric acid and hydriodic acid. It should be pointed out that a great practical advantage in the use of methylated sugars lies in the ease and rapidity with which methoxyl groups can be estimated by Zeisel's method, or a micro modification of it, the substance being heated with hydriodic acid (S.G. 1·7) in a stream of carbon dioxide, and the liberated methyl iodide precipitated as silver iodide, or estimated volumetrically. Ethoxyl groups can, of course, be estimated in a similar way, but sugar ethyl ethers have not been extensively used in structural determinations.



α-Methylglucopyranoside Tetramethyl α-methylglucopyranoside Tetramethyl α-D-glucopyranose

In the above and in later cyclic formulae the hydrogen atoms directly attached to the rings are omitted for the sake of clarity.

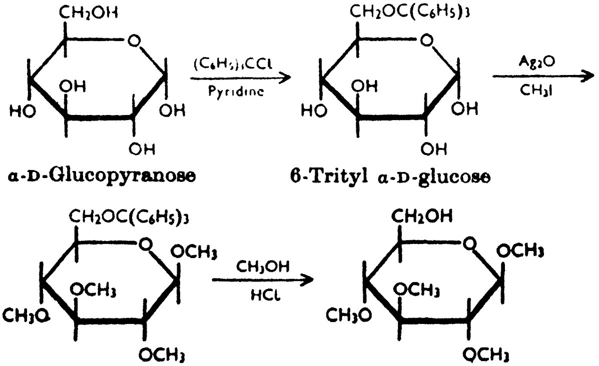
The Unequal Reactivity of the Hydroxyl Groups. In a sugar such as β-D-glucopyranose, the hydroxyl groups are not equally reactive. The incipient reducing group on C_1 is obviously the most readily substituted, but even in the glycosides, there appear to be distinct differences in reactivity between the remaining four hydroxyl groups. Thus when either α- or β-methylglucoside are treated with thallium hydroxide followed by methylation with methyl iodide, in addition to the fully methylated derivatives, a mixture of all three possible trimethyl methylglucosides is obtained in which the 2,4,6-trimethyl methylglucosides (60%) preponderate and the 2,3,4-trimethyl methylglucosides are only present in small amounts.* It would be expected

* Barker, Hirst and J. K. N. Jones, *J.C.S.*, 1938, 1695.

that the primary alcoholic groups would be more easily methylated than the secondary alcoholic residues and this appears to be so, for it has been observed also in experiments on the methylation of potassium hydroxide addition compounds of α- and β-methylglucosides,* and furthermore selective methylation of this type has been noted with a number of disaccharides. At the same time there are also differences in reactivity between the individual secondary hydroxyl groups. This appears not only in the methylation of the methylglucosides with thallium mentioned above, but also in the absence of a primary alcoholic group, for methylxylosides† on treatment with thallous hydroxide followed by methyl iodide gave a mixture of 2,3-, 3,4-, and 2,4-dimethyl methylxylosides, the latter in the highest yield.

Triphenylmethyl Ethers

The outstanding reactivity of the primary alcoholic group in free sugars and glycosides is shown in the formation of the triphenylmethyl (trityl) ethers. In all cases where a primary hydroxyl group is available, substitution takes place in that position when the sugar‡ or its derivative is treated with triphenylchloromethane in pyridine solution. This reaction was thought at one time to be diagnostic for the presence of primary alcoholic groups until it was shown that α-methyl-L-fucoside, a compound containing no primary alcoholic group, gave a crystalline monotrityl ether.§ Nevertheless the trityl



α-D-Glucopyranose

6-Trityl α-D-glucose

2,3,4-Trimethyl β-methylglucopyranoside

---

* Heddle and Percival, *J.C.S.*, 1938, 1690.

† Barker, Hirst and J. K. N. Jones, *J.C.S.*, 1946, 783.

‡ Helferich, *Z. Angew. Chem.*, 1928, **41**, 871.

§ Hockett and Hudson, *J. Amer. Chem. Soc.*, 1934, **56**, 945.

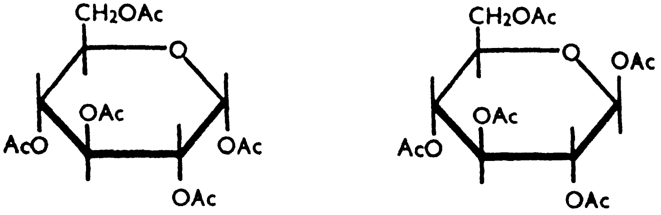
compounds are exceedingly useful in synthetic work since the ether residue is readily removed either by dilute acid or by hydrogenation (hydrogenolysis). The foregoing preparation of 2,3,4-trimethyl β-methylglucoside illustrates this point.[*] Such partly methylated sugars are of great importance as reference compounds for comparison with fragments obtained from the hydrolysis of methylated di- and poly-saccharides, as will be shown later.

Esterification

Acetylation

The hydroxyl groups in sugars and their derivatives are easily protected by acetylation; either by treatment with acetic anhydride and pyridine at room temperature or heating with acetic anhydride and sodium acetate. The compounds so obtained are not suitable for structural studies since the acetyl groups are fairly easily hydrolysed by acids as well as alkalis. Nevertheless sugar acetates have important applications notably in the preparation of the acetohalogenoses.

Variations in the conditions of acetylation give different isomers. When glucose is heated with sodium acetate and acetic anhydride β-D-glucose pentaacetate results,[†] whereas at 0° in the presence of zinc chloride the α-form is obtained.[‡]



(α) D-Glucose pentaacetates (β)

Some other Acylations

Other aliphatic esters of sugars are made in an analogous fashion, but esters of aromatic and sulphonic acids are prepared using the acyl chloride and pyridine. In this way α-pentabenzoyl glucopyranose can be obtained.

---

[*] Haworth, Hirst, Miller and Learner, *J.C.S.*, 1927, 2443.

[†] Tanret, *Bull. soc. chim.*, 1895, **13**, 261.

[‡] Hudson and Dale, *J. Amer. Chem. Soc.*, 1915, **37**, 1264.

Sulphonic esters, especially the *p*-toluenesulphonates, are of interest for two main reasons. In the first case their hydrolysis with alkali can give rise to anhydro sugars (Chapter IV) and secondly they give a reliable method for showing the presence of primary alcoholic groups in a given derivative, and also for converting it into a deoxy sugar. Oldham and Rutherford* showed that when a sugar derivative having a *p*-toluenesulphonic ester residue on a primary, but not on a secondary alcoholic group, is heated with sodium iodide in acetone solution, sodium *p*-toluenesulphonate is produced quantitatively, and iodine replaces the tosyl residue. This iodo-compound on reduction may then be converted into the ω-deoxy sugar (see page 200). Galactose derivatives can be transformed into fucose derivatives in this way, and D-rhamnose has been prepared from 2,3,4-tribenzoyl α-methyl-D-mannoside 6-*p*-toluenesulphonate.†

Reactions Involving Two Hydroxyl Groups Simultaneously

Condensation with Acetone

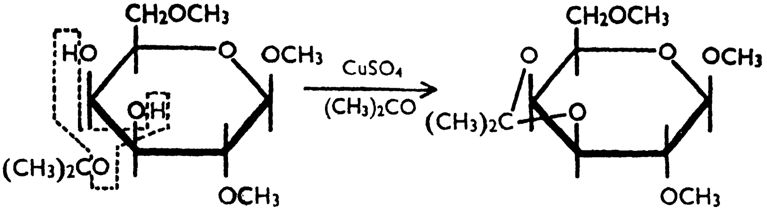
E. Fischer discovered that glucose would condense with acetone to form diacetone glucose, or, more correctly, di*iso*propylidene glucose. This beautifully crystalline compound can be made readily by shaking glucose with acetone containing a little sulphuric acid, hydrochloric acid, or copper sulphate. In this reaction, which is a general one, two suitably placed hydroxyl groups of the sugar react with the acetone with the elimination of water. These *iso*propylidene compounds are not, as a rule, suitable for structural determinations since the ring system of the sugar may change in order to accommodate the *iso*propylidene residues which enter. They have, nevertheless, rendered service in the preparation of partly methylated reference sugars, and, to a limited extent, may be used to determine whether adjacent hydroxyl groups are *cis* to one another. Thus the view that a dimethyl galactose isolated from a methylated seaweed polysaccharide‡ was 2,6-dimethyl galactose was given strong support since the dimethyl β-methylgalactoside prepared therefrom condensed readily with acetone in the presence of copper sulphate, and since in cyclic compounds it is *cis* hydroxyl groups which con-

---

* *J. Amer. Chem. Soc.*, 1932, **54**, 366.

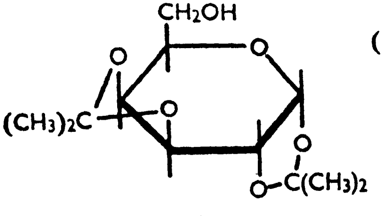
† Haskins, Hann and Hudson, *J. Amer. Chem. Soc.*, 1946, **68**, 628.

‡ Dewar and Percival, *J.C.S.*, 1947, 1622.

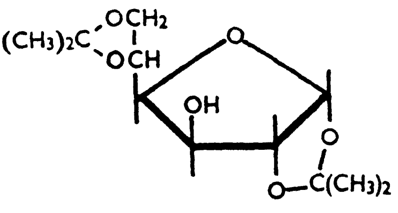


Condensation of 2,6-Dimethyl β-methylgalactoside with acetone

dense with acetone the only two possibilities in this case were those on C_3 and C_4. The special property of acetone compounds is that they are stable to alkali, but are readily hydrolysed by dilute acids, and the determination of the exact constitution of some of them has been a matter of difficulty. Aldohexoses, with five hydroxyl groups available, can react with two acetone molecules provided two pairs of *cis* hydroxyl groups are present; if these can be produced by changing the ring structure from the pyranose to the furanose form, this too occurs. With a sugar like galactose this is not necessary, and the diacetone galactose prepared by shaking the sugar with acetone containing hydrogen chloride is the 1,2-3,4-diacetone derivative.* But this is not so for glucopyranose which can only give



Diacetone galactopyranose



Diacetone glucofuranose

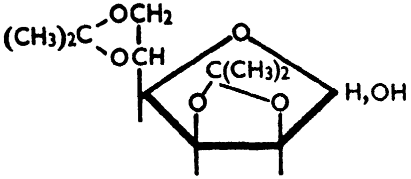
a diacetone derivative, 1,2-5,6-diacetone glucofuranose,† by changing to the furanose form. Mannopyranose similarly undergoes rearrangement, but in this case the hydroxyl group on C_1 is left free.‡ Fructopyranose which can condense with acetone in two ways, retains its pyranose structure in both the diacetone fructoses.†

---

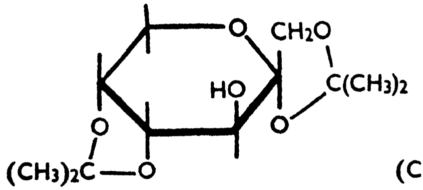
* Freudenberg and Smeykal, *Ber.*, 1926, **59**, 100.

† Anderson, Charlton and Haworth, *J.C.S.*, 1929, 1329; 1337.

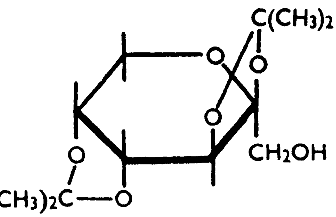
‡ Goodyear and Haworth, *J.C.S.*, 1927, 3136.



Diacetone mannofuranose

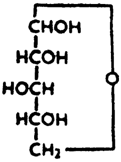


α-Diacetone fructose

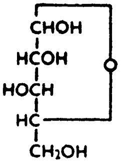


β-Diacetone fructose

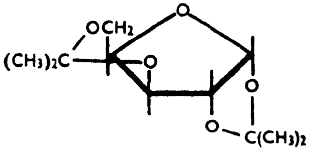
Diacetone xylose is an interesting compound. Obviously two pairs of *cis* hydroxyl groups could not be provided by xylopyranose, and xylofuranose, if it existed, would seem even less likely to provide them. Nevertheless a diacetone xylose is obtained, the 1,2-3,5-diacetone derivative[*] since in the actual model the hydroxyl groups on C_3 and C_5 are so disposed that they are available for condensation with acetone to form a six-membered ring.



D-Xylopyranose



D-Xylofuranose



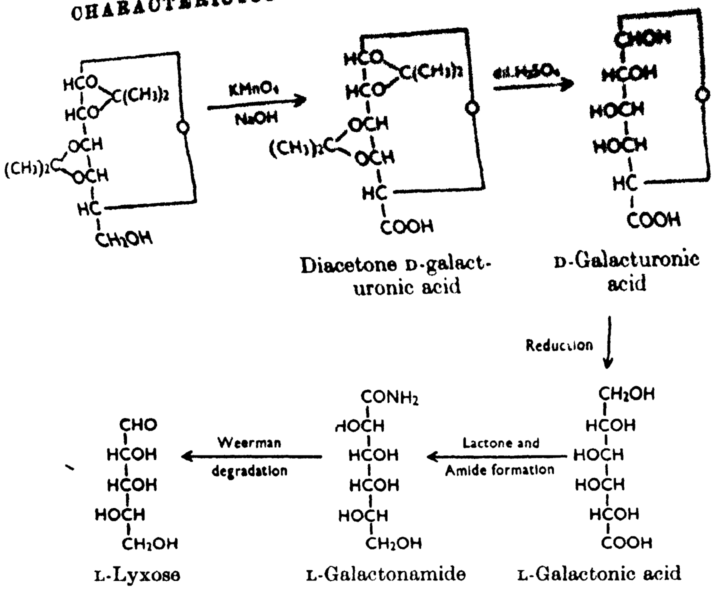
Diacetone xylose

The diacetone derivatives of the hexoses are very useful for preparing monomethyl sugars and other monosubstituted derivatives such as phosphates and sulphates. Thus diacetone glucose gives 3-methyl glucose on methylation and hydrolysis, and diacetone galactose gives 6-methyl galactose. Diacetone galactose was used to enter the L-series in the synthesis of ascorbic acid,[†] the key compound in the synthesis being L-xylosone for which L-xylosazone was needed. The steps were as follows:

---

[*] Haworth and Porter, *J.C.S.*, 1928, 611.

[†] Ault, Baird, Carrington, Haworth, Herbert, Hirst, Percival, Smith and Stacey, *J.C.S.*, 1933, 1419.



Diacetone D-galacturonic acid

D-Galacturonic acid

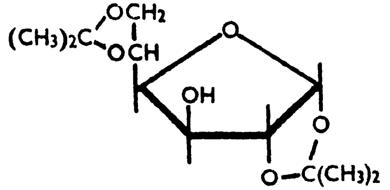
L-Galactonic acid

L-Galactonamide

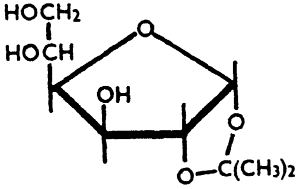
L-Lyxose

Diacetone galactose can be converted into a 6-tosyl derivative, from which, as mentioned in connexion with " tosyl " esters, diacetone D-fucose can be obtained *via* the iodide by reduction.

In the diacetone hexoses the acetone residues attached to the potential reducing group are more firmly attached than the others. By treatment with dilute (N/10) mineral acid, 1,2-5,6-diacetone glucofuranose is converted at room temperature into 1,2-monoacetone glucose with retention of the furanose ring since, on methylation and hydrolysis, 3,5,6-trimethylglucose is obtained. It is by such methods involving the preparation and identification of methylated sugars that the structures of the acetone compounds mentioned above have been determined.*



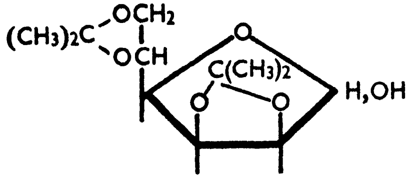
Diacetone glucofuranose



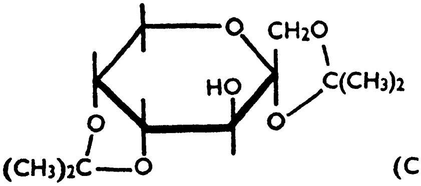
1,2-Monoacetone glucofuranose

---

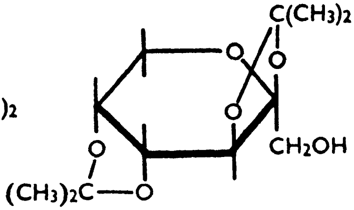
* *The Constitution of Sugars*, 1928, Arnold, London.



Diacetone mannofuranose

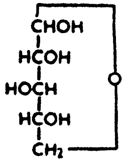


α-Diacetone fructose

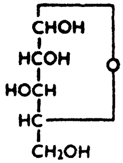


β-Diacetone fructose

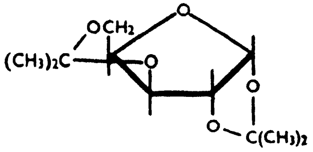
Diacetone xylose is an interesting compound. Obviously two pairs of *cis* hydroxyl groups could not be provided by xylopyranose, and xylofuranose, if it existed, would seem even less likely to provide them. Nevertheless a diacetone xylose is obtained, the 1,2-3,5-diacetone derivative* since in the actual model the hydroxyl groups on C_3 and C_5 are so disposed that they are available for condensation with acetone to form a six-membered ring.



D-Xylopyranose



D-Xylofuranose



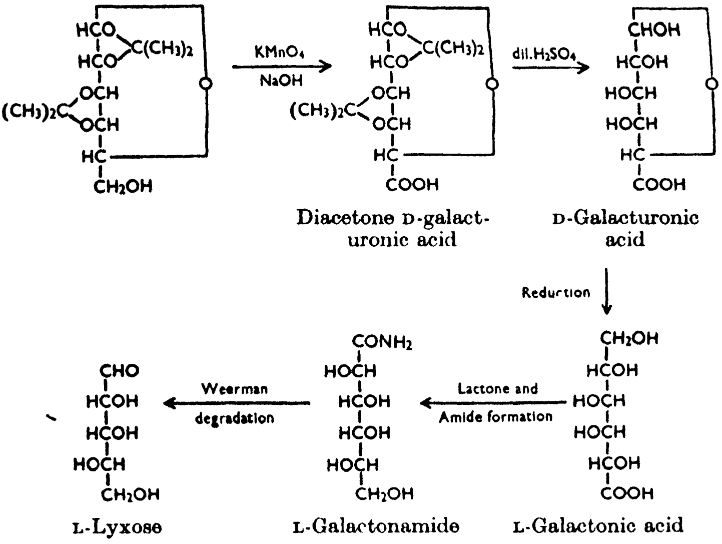
Diacetone xylose

The diacetone derivatives of the hexoses are very useful for preparing monomethyl sugars and other monosubstituted derivatives such as phosphates and sulphates. Thus diacetone glucose gives 3-methyl glucose on methylation and hydrolysis, and diacetone galactose gives 6-methyl galactose. Diacetone galactose was used to enter the L-series in the synthesis of ascorbic acid,† the key compound in the synthesis being L-xylosone for which L-xylosazone was needed. The steps were as follows:

---

* Haworth and Porter, *J.C.S.*, 1928, 611.

† Ault, Baird, Carrington, Haworth, Herbert, Hirst, Percival, Smith and Stacey, *J.C.S.*, 1933, 1419.



Diacetone D-galacturonic acid

D-Galacturonic acid

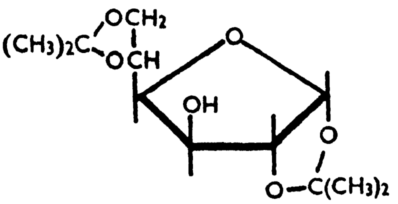
L-Lyxose

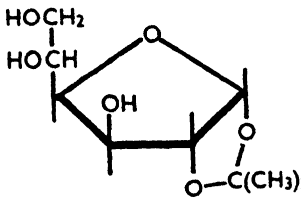
L-Galactonamide

L-Galactonic acid

Diacetone galactose can be converted into a 6-tosyl derivative, from which, as mentioned in connexion with " tosyl " esters, diacetone D-fucose can be obtained *via* the iodide by reduction.

In the diacetone hexoses the acetone residues attached to the potential reducing group are more firmly attached than the others. By treatment with dilute (N/10) mineral acid, 1,2-5,6-diacetone glucofuranose is converted at room temperature into 1,2-monoacetone glucose with retention of the furanose ring since, on methylation and hydrolysis, 3,5,6-trimethylglucose is obtained. It is by such methods involving the preparation and identification of methylated sugars that the structures of the acetone compounds mentioned above have been determined.[*]


Diacetone glucofuranose

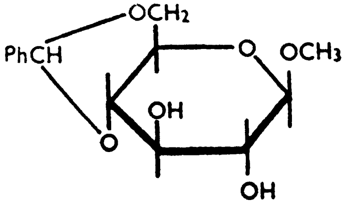

1,2-Monoacetone glucofuranose

---

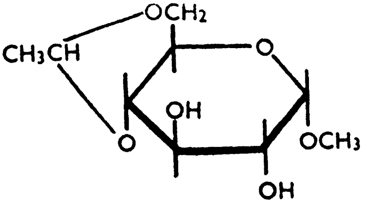
[*] *The Constitution of Sugars*, 1928, Arnold, London.

Condensation with Aldehydes

Sugars also condense with aldehydes; with formaldehyde to form methylene compounds, with acetaldehyde to yield ethylidene compounds and with benzaldehyde. With the last and zinc chloride, 1,2-monoacetone glucofuranose gives the 3,5-benzylidene derivative at first, but on heating the 5,6-derivative is produced.* From the former compound by oxidation and hydrolysis D-glucuronic acid may be prepared (page 152). Glucose with formaldehyde gives methylene glucoses, with paraldehyde and sulphuric acid 4,6-ethylidene glucose, and with benzaldehyde gives 4,6-benzylidene glucose. Similar 4,6-substituted derivatives are obtained with the α- and β-methylglucopyranosides, and it is clear that aldehydes do not require adjacent *cis* hydroxyl groups before condensation can take place. It should also be noted that the compounds obtained by condensation with aldehydes, except formaldehyde, contain an additional asymmetric centre.



4,6-Benzylidene β-methyl glucoside



4,6-Ethylidene α-methyl glucoside

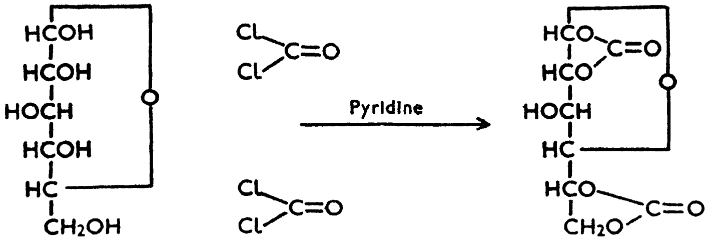
The Sugar Carbonates and the Crystalline Alkylfuranosides

Sugar carbonates should properly have been discussed under esters, but since their structures are similar to the acetone compounds, we have deferred considering them until now. In the sugar carbonates, which are usually prepared by the agency of carbonyl chloride and pyridine on the sugar or its derivative, adjacent *cis* hydroxyl groups are esterified. Thus glucose reacts to give a furanose derivative just as in the condensation with acetone. Since the carbonates are esters, they are readily hydrolysed by alkali; on the other hand they are, unlike the acetone compounds, relatively stable to acids, and this difference of behaviour was exploited in an ingenious way by Sir W. N. Haworth and his co-workers.† On treating 1,2-monoacetone glucofuranose (1) with carbonyl chloride, or glucose in acetone with carbonyl chloride, glucose 1,2-monoacetone 5,6-

---

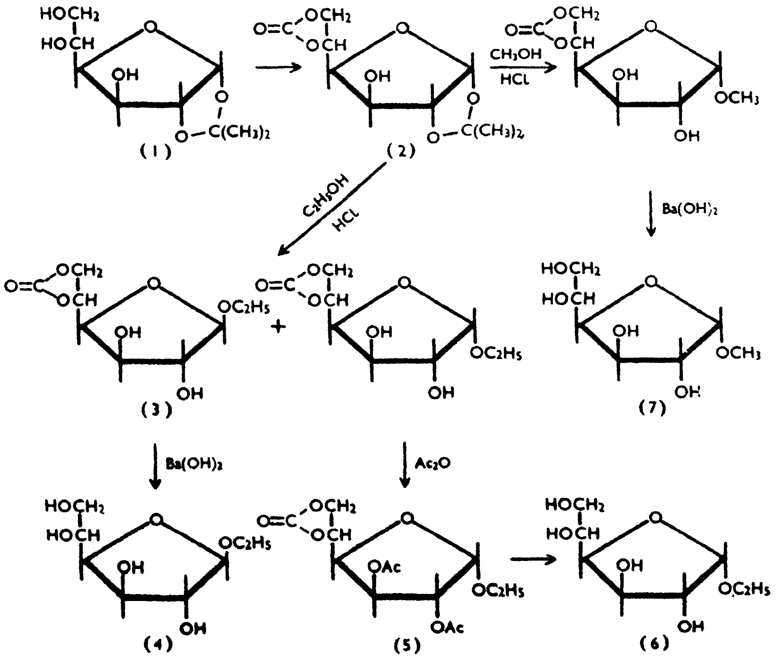
* Brigl and Grüner, *Ber.*, 1932, 65, 1428.

† *J.C.S.*, 1929, 2796; 1930, 649; 1932, 2254.

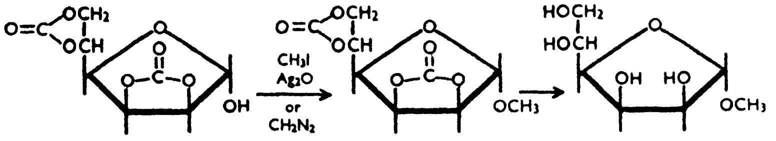


Glucose 1,2-5,6-dicarbonate

carbonate (2) was obtained. When this substance was treated with ethanolic hydrogen chloride, crystalline β-ethylglucofuranoside 5,6-carbonate (3) was obtained, which on treatment with barium hydroxide gave crystalline β-ethylglucofuranoside (4), and it was also possible by acetylation to isolate both the α- (5) and β-forms of 2,3-diacetyl ethylglucofuranoside 5,6-carbonate in the crystalline state. On deacetylation of the α-form crystalline α-ethylglucofuranoside (6) was obtained. By treating glucofuranose 1,2-monoacetone 5,6-carbonate (2) with methanolic hydrogen chloride followed by removal of the carbonate residue with alkali, crystalline α-methylglucofuranoside (7) was also obtained.



β-Ethylglucofuranoside α-Ethylglucofuranoside α-Methylmannofuranoside was obtained as a crystalline product from mannofuranose 2,3-5,6-dicarbonate (8) by methylation, either with methyl iodide and silver oxide, or diazomethane, followed by hydrolysis with barium hydroxide. Once this substance had been prepared in the Birmingham laboratories it caused "γ"-methylmannoside, prepared by Fischer's method as a syrup, to crystallise even without intentional nucleation.* Complete methylation followed by hydrolysis and oxidation gave crystalline γ-tetramethyl D-mannonolactone, thus proving the structure.



(8) α-Methylmannofuranoside

Sugar Halohydrins

A very outstanding group of compounds is obtained when one or more of the hydroxyl groups in a sugar are replaced by a halogen atom. A particular example has been mentioned already, the 6-iodide prepared from the tosyl esters.

The Acetohalogenoses

Of this group, by far the most important are the compounds in which the halogen atom replaces the hydroxyl in the potential reducing group of the sugar, and where the remaining free hydroxyl groups are protected by acetyl residues. Compounds of this type find extensive application in the preparation of glycosides, disaccharides, glycoseens, glycals and deoxy sugars.

Tetraacetyl α-Glucosyl 1-Bromide and its Reactions

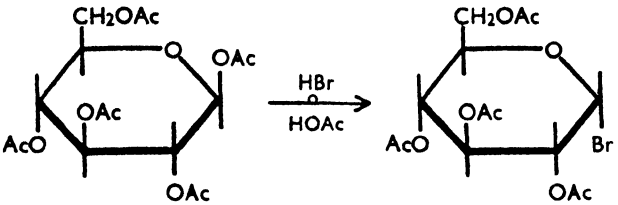
By the action of anhydrous hydrogen bromide in glacial acetic acid on β-glucose pentaacetate, Fischer† prepared the compound usually called α-acetobromoglucose; more correctly described as tetraacetyl α-glucosyl 1-bromide, which had been made ten years earlier‡ by the interaction of glucose and acetyl bromide.

---

* Haworth, Hirst and Webb, *J.C.S.*, 1930, 651.

† *Ber.*, 1911, **44**, 1898.

‡ Königs and Knorr, *Ber.*, 1901, **34**, 957.

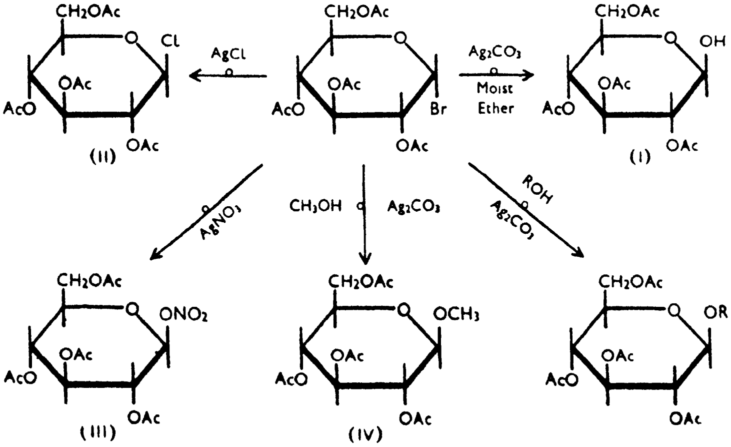


β-Glucose pentaacetate   Acetobromoglucose

The corresponding 1-chloro- and 1-fluoro-compounds and many similar α-acetobromo compounds have been made using other sugar acetates prepared, for example, from mannose, galactose and the pentoses, but the reactions of acetobromoglucose are typical of the class and are summarised below.

Simple Replacement Reactions

When acetobromoglucose is shaken with silver carbonate in moist ether[*] 2,3,4,6-tetraacetyl β-glucose (I) is produced. On shaking with silver chloride in ether tetraacetyl β-glucosyl 1-chloride (β-acetochloroglucose) (II) is obtained[†] and with silver nitrate β-acetonitroglucose (III) is isolated. It will be observed that in all these reactions Walden inversion has taken place; this is usual in reactions with silver derivatives. The most outstanding of all these replacement reactions results in the formation of glycosides. With methanol and silver carbonate, α-acetobromoglucose gives tetraacetyl β-methylglucopyranoside (IV).[‡] Here again Walden inversion has



---

[*] Fischer and Delbrück, *Ber.*, 1909, **42**, 2778.

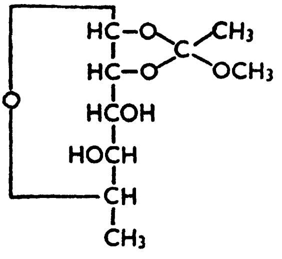
[†] Schlubach *et al.*, *Ber.*, 1928, **61**, 287.

[‡] Königs and Knorr, *Ber.*, 1901, **34**, 957.

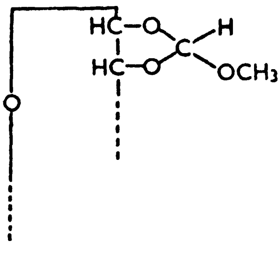
taken place. This reaction is quite general and is of great value in preparing β-glucopyranosides, which are obtained by deacetylating the tetraacetates. Thus Königs and Knorr obtained β-phenylglucoside employing potassium phenate, and an immense number of new glycosides as well as many naturally occurring ones have been synthesised in this way. Since disaccharides are really sugars joined by glycosidic linkages, it is not surprising that α-acetobromoglucose and similar compounds of this type have been used in their synthesis (see Chapter V). Condensations with sulphides to give sulphur derivatives, with the silver salts of some of the purines to give nucleosides, and with thiourethane derivatives to give mustard oil glycosides may be mentioned also.

Orthoester Structures

A novel type of cyclic structure is to be found in certain acetylated glycosides, prepared by the action of quinoline in the presence of methanol* on certain acetobromosugars. In these products the curious fact emerges that one acetyl group is not removed by alkali. However after the glycosidic residue is removed, a process which takes place very rapidly indeed even with very dilute acid, this remaining acetyl group is removed in the normal way by alkali, although previously it was quite stable. Examples of this phenomenon are to be found in a monoacetyl methylrhamnoside† and the corresponding methylmannoside; it is explained by the acetyl group on C_2 linking up with the glycosidic methoxyl on C_1 to give an orthoester or an α-methoxy ethylidene compound.



Methylrhamnoside orthoacetate



Orthoacetate

In the case of the monoacetyl methylmannoside, methylation followed by hydrolysis gave 3,4,6-trimethyl mannose, the substance used with boric acid in the conductivity experiments in the last chapter. The isolation of this compound proves that the orthoester is a pyranose derivative in spite of the extreme ease of hydrolysis of the glycosidic methoxyl with dilute acid. Pacsu* has shown that in certain compounds, for example in acetates of the disaccharide turanose, the orthoester structure can be present, the acetyl group linking with the free " reducing " hydroxyl group in the acetylated sugar as distinct from an acetylated glycoside.

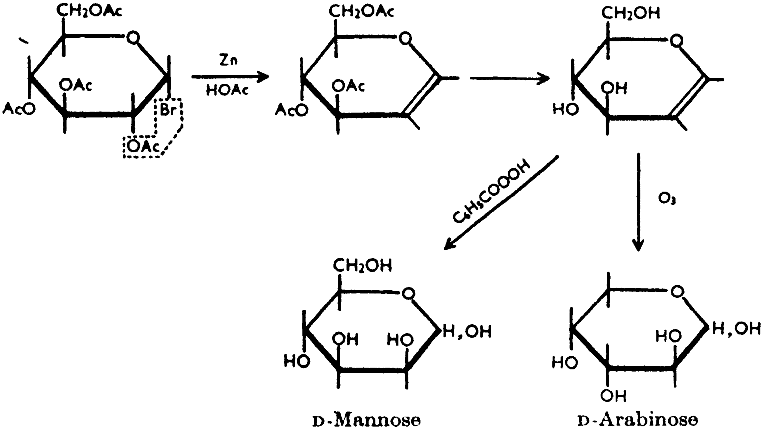
---

* Fischer, Bergmann and Rabe, *Ber.*, 1920, **53**, 2362.

† Bott, Haworth and Hirst, *J.C.S.*, 1930, 1395.

The Preparation of Glycals and Deoxy Sugars

α-Acetobromoglucose undergoes an interesting reaction on treatment with zinc and acetic acid: the elements of acetyl bromide are removed with the production of an unsaturated compound triacetyl glucal† which on deacetylation yields glucal. This material on treatment with ozone gives arabinose‡ proving the double bond to lie between C_1 and C_2. When glucal is treated with perbenzoic acid in the presence of methanol it yields α-methylmannoside,§ and with perbenzoic acid and water it gives mannose.|| Clearly with the double bond between C_1 and C_2, glucal is as equally a mannose as a glucose derivative. Although glucal itself on treatment with perbenzoic acid gives no detectable glucose derivatives, trimethyl glucal which was proved to have a pyranose structure|| gives mainly derivatives of glucose.



---

* *J. Amer. Chem. Soc.*, 1932, **54**, 3649. See *Advances in Carbohydrate Chemistry*, 1945, **1**, 78.

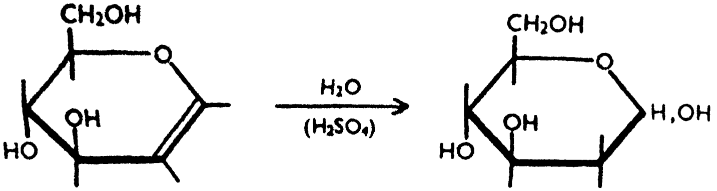
† Fischer, *Ber.*, 1914, **47**, 196.

‡ Fischer, Bergmann and Schotte, *Ber.*, 1920, **53**, 509.

§ Bergmann and Schotte, *Ber.*, 1921, **54**, 1564.

|| Hirst and Woolvin, *J.C.S.*, 1931, 1131.

Glucal is a representative of the class known as glycals, of interest since with dilute mineral acids they are transformed into 2-deoxy or 2-desoxy sugars. These substances are found in nature, for example 2-deoxy ribose in animal nucleic acid, where, instead of a >CHOH group in the sugar chain in the 2-position there is a $>CH_2$ group.

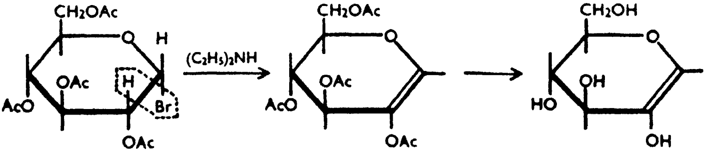


Glucal 2-Deoxy-D-glucose

When glucal is treated with dilute sulphuric acid 2-deoxy glucose is obtained. 2-Deoxy glucose in general properties is more reactive than glucose; it restores the colour to Schiff's reagent and imparts a green colour to a pine chip in the presence of hydrogen chloride. Of special interest is the ease with which 2-deoxy glucose (or 2-glucodesose as it is also called), reacts with dilute methanolic hydrogen chloride. The glycoside (glucodesoside) is formed exceedingly rapidly and is equally easily hydrolysed, in spite of the fact that it appears to have a pyranose structure.*

Glycoseens

By the action of diethylamine on α-acetobromoglucose, the elements of hydrogen bromide are removed and another unsaturated compound is obtained, tetraacetyl glucoseen-(1,2), deacetylation giving glucoseen-(1,2). This substance on oxidation with alkaline



D-Glucoseen (1,2)

potassium permanganate is degraded to D-arabonic acid, so that here we have a further method for descending the series. Sugar derivatives such as the glycals, 1,2-glycoseens, osazones and non-terminal deoxy sugars with fewer asymmetric carbon atoms than the parent sugars from which they are usually named, may be described more logically with reference to the remaining asymmetric atoms,* e.g. D-glucal is D-*arabo*hexal, D-glucosazone is D-*arabo*-hexosazone.

---

* Levene and Mikeska, *J. Biol. Chem.*, 1930, 88, 791.

Another glucoseen, glucoseen-(5,6), is made from 1,6-dibromoglucose triacetate. These glycoseens although they may be regarded as anhydro sugars are not usually classified as such.

---

* Sowden, *J. Amer. Chem. Soc.*, 1947, **69**, 1047.

CHAPTER IV

SUGAR ANHYDRIDES AND AMINO SUGARS

IT is not surprising that, with their numerous hydroxyl groups, it is possible to convert sugars by the loss of the elements of water into anhydro compounds. These substances are worthy of attention* because of their value in interconversion reactions, and their utilisation in the synthesis of disaccharides and amino sugars. Anhydrides of various types are known, their structures depending on the method of ring formation.

Anhydrides of the Glycosan Type

When reducing sugars are heated in the dry state under reduced pressure, mixtures of products are obtained, usually called glycosans. More definite products are obtained when polysaccharides such as starch† or ivory nut mannan‡ are subjected to similar treatment. The product obtained from starch is laevoglucosan or β-glucosan, a mannosan is obtained from ivory nut, and the vacuum pyrolysis of lactose gives a mixture of β-glucosan and β-galactosan. Compounds of the β-glucosan type are easily prepared also, by the alkaline hydrolysis of β-phenylglucosides, in fact laevoglucosan was first prepared by Tanret§ by treating natural phenolic glycosides, such as salicin, with alkali. Hudson and his co-workers|| have shown that β-phenylglucoside gives β-glucosan, β-phenylgalactoside gives β-galactosan, that mannosan may be obtained likewise, and that the method is also applicable to the disaccharides.¶

β-Glucosan is a crystalline substance, $[\alpha]_D - 66°$, of the molecular formula, $C_6H_{10}O_5$, which is non-reducing to Fehling's solution and is not fermented by yeast, but on heating with dilute acids it is converted into glucose. Karrer** synthesised β-glucosan from acetobromoglucose and trimethylamine, when the tetraacetyl glucosido-

---

* See Peat, *Advances in Carbohydrate Chemistry*, 1947, **2**, 37.

† Zemplén and Gerecs, *Ber.*, 1931, **64**, 1545.

‡ Knauf, Hann and Hudson, *J. Amer. Chem. Soc.*, 1941, **63**, 1447.

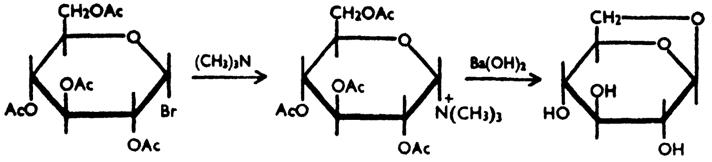
§ *Compt. rend.*, 1894, **119**, 158.

|| *J. Amer. Chem. Soc.*, 1942, **64**, 1483.

¶ *J. Amer. Chem. Soc.*, 1943, **65**, 3, 1848.

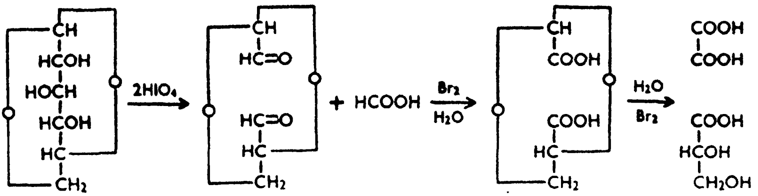
** Karrer and Smirnoff, *Helv. Chim. Acta*, 1921, **4**, 819.

trimethylammonium bromide on treatment with barium hydroxide gave β-glucosan.



β-Glucosan

The constitution of β-glucosan has been proved by several methods: methylation and hydrolysis give 2,3,4-trimethyl glucose.* Periodate oxidation† also proves its structure, as well as that of the other hexosans of the same type. Two molecules of periodic acid are required for the oxidation, one molecule of formic acid is liberated together with one molecule of L′-oxy-D-methylene-diglycollic aldehyde. The structure of this latter product follows from its oxidation to L′-oxy-D-methylene diglycollic acid, which on further oxidative hydrolysis is broken down to oxalic acid and D-glyceric acid (cf. page 42) the acids being isolated as their strontium salts.



β-Glucosan L -Oxy-D-methylene diglycollaldehyde L′-Oxy-D-methylene diglycollic acid

It is clear that β-glucosan is really an internal glucoside, so that its reversion to glucose on hydrolysis with mineral acids is explained.

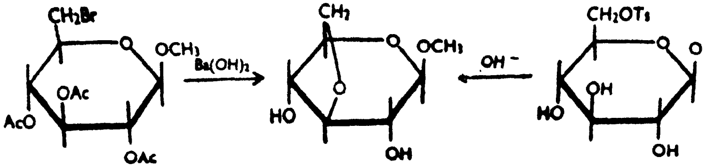
3,6-*Anhydrides. The Pentaphan or Hydrofuranol Ring*

The anhydrides of the glycosan type just mentioned have special properties since the anhydro ring is glycosidic in character. In the second group of anhydrides with which we shall deal, however, the anhydro ring becomes of more importance than the pyranose ring and influences directly the properties of the 3,6-anhydrides. By treating triacetyl β-methylglucoside 6-bromohydrin with barium hydroxide, Fischer and Zach* obtained 3,6-anhydro β-methylglucoside. This compound is prepared more conveniently from 6-tosyl β-methylglucopyranoside† by the action of alkali, and also from the corresponding 6-sulphate‡ and 6-nitrate.§ Similarly 3,6-anhydro-α-methylgalactoside has been prepared from the corresponding 6-tosyl derivative,|| the reaction being quite general.

---

* Irvine and Oldham, *J.C.S.*, 1921, 1744.

† Jackson and Hudson, *J. Amer. Chem. Soc.*, 1940, **62**, 958.



3,6-Anhydro β-methyl glucoside

Haworth and Smith† made a careful study of the properties of 3,6-anhydro-glucose and -galactose, the free sugars being readily prepared by the hydrolysis of the methylglycosides. The free 3,6-anhydro sugars, as Fischer and Zach had noted, are unusual in that they restore the colour to Schiff's reagent. On treatment with methanolic hydrogen chloride either at room temperature or on heating it is extraordinary that in neither case is the anhydromethylhexopyranoside regenerated; from 3,6-anhydro-glucose the methylglucofuranoside and from 3,6-anhydro-galactose† a dimethyl acetal are produced. Indeed unless the pyranoside ring is present before the 3,6-anhydro ring is formed, it is impossible to prepare the 3,6-anhydro-methylhexopyranosides. In the case of 3,6-anhydro-glucose, the furanoside is more stable than the pyranoside, and if a trace of acid is allowed to come into contact with 3,6-anhydro-α-methylglucopyranoside, it is converted at once into the corresponding furanoside, the change taking place without the loss of the glycosidic methyl group.† Two five-membered rings fused together are evidently in these circumstances more stable than a five- and a six-membered ring. The same conversion of pyranoside to furanoside cannot take place in the galactose series since the two rings would interfere with

---

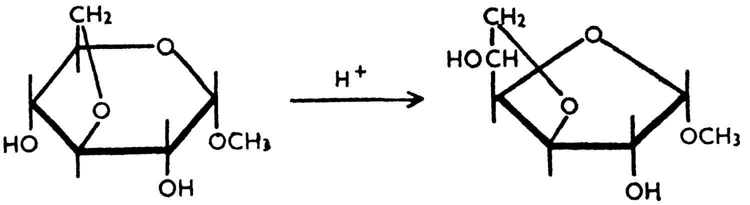
* *Ber.*, 1912, **45**, 456.

† Haworth, Owen and Smith, *J.C.S.*, 1941, 88; Haworth, Jackson and mith, *ibid.*, 1940, 620.

‡ Duff and Percival, *J.C.S.*, 1941, 830.

§ Gladding and Purves, *J. Amer. Chem. Soc.*, 1944, **66**, 76, 153.

|| Ohle and Thiel, *Ber.*, 1933, **66**, 525.



one another because of the reversal of H and OH on C_4; this can be demonstrated readily with the use of models. It is evident therefore that the 3,6-anhydro (hydrofuranol or pentaphan) ring which is very stable both to acid and alkaline hydrolysis and to oxidising agents, is the governing factor determining the stability of these compounds.

An anhydride of an unusual type containing two six-membered rings has been isolated by the deacylation of 2,3,4-tribenzoyl α-methyl altroside.* The product consumed one equivalent of periodate and neither formaldehyde nor formic acid was released. It is evident, therefore, that two adjacent hydroxyl groups exist in the compound and the 3,6-anhydride formulation is excluded. Its formulation as 2,6-anhydro α-methyl altroside is confirmed also by hydrogenation to yield a 1,5-anhydro hexahydric alcohol (1,5-anhydro D-talitol). It may be noted that the hydroxyl group which participates in anhydride formation, that on C_2, is on the left hand side of the projection formula and that the same is true of the hydroxyl groups on C_3 in the corresponding glucose, galactose and mannose derivatives which give 3,6-anhydrides.

Anhydrides of the Ethylene Oxide Type

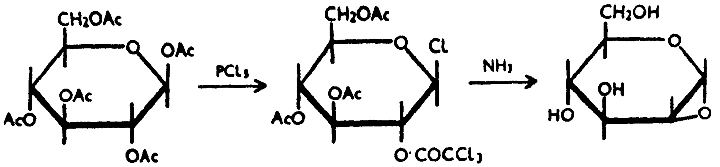
Undoubtedly the most reactive class of sugar anhydrides is that related to ethylene oxide, where adjacent carbon atoms are united by an oxygen bridge. The fact that the formation and disruption of these compounds is accompanied by Walden inversions makes possible many transformations and interconversions, one of the most important results of which is to throw light on the constitution of the naturally occurring amino sugars.

The first compound of this class to be isolated,† 1,2-anhydro D-glucose, is a special case inasmuch as it is also a glucosan; it is prepared by the action of ammonia on the product of interaction between phosphorus pentachloride and glucose pentaacetate, the latter reaction introducing a trichloroacetyl group on C_2.

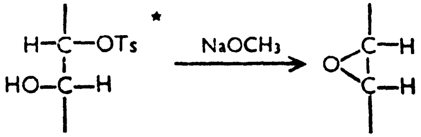
---

* Rosenfeld, Richtmyer and Hudson, *J. Amer. Chem. Soc.*, 1948, **70**, 2201.

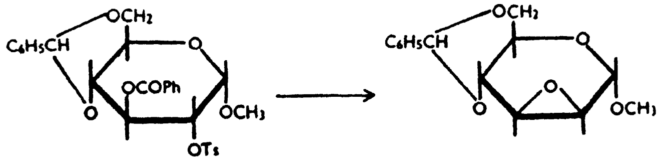
† Brigl, *Z. physiol. chem.*, 1921, **116**, 1; 1922, **122**, 245.



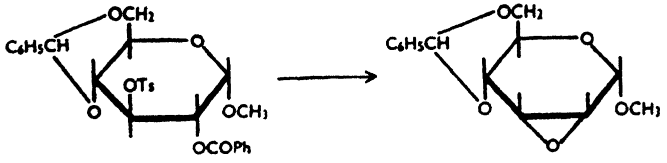
The usual method of introducing ethylene oxide rings into a sugar is by the hydrolysis of *p*-toluenesulphonates (see page 54) or methanesulphonates, generally by the action of sodium methoxide in the cold. Hydrolysis of a *p*-toluenesulphonate gives an ethylene oxide ring compound if there is, on the adjacent carbon atom, a hydroxyl group *trans* to the *p*-toluenesulphonyl group. When this state of affairs



exists, a Walden inversion occurs, as shown, on the carbon atom which formerly carried the *p*-toluenesulphonyl residue. Thus 2-tosyl 3-benzoyl 4,6-benzylidene α-methylglucoside (I) gives 2,3-anhydro 4,6-benzylidene α-methylmannoside (II) and 2-benzoyl 3-tosyl 4,6-benzylidene α-methylglucoside (III) gives 2,3-anhydro 4,6-benzylidene α-methylalloside† (IV). If the groups in question are *cis*, or there are no free hydroxyl groups available at all as in 3-tosyl diacetone glucose, then the sulphonic ester grouping is very difficult to remove on treatment with alkali, and neither anhydride formation nor Walden inversion takes place.



(I) (II)



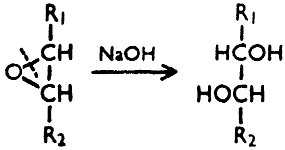
(III) (IV)

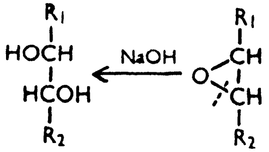
---

* Ts = tosyl = *p*-toluenesulphonyl.

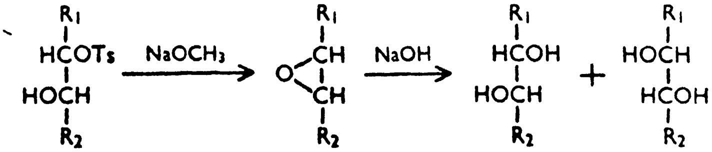
† G. J. Robertson and Griffith, *J.C.S.*, 1935, 1193.

The main interest in ethylene oxide derivatives lies in their behaviour on treatment with alkalis. The ring opens, with another Walden inversion, and two products, depending on which oxygen bridge is broken, may result from this scission.



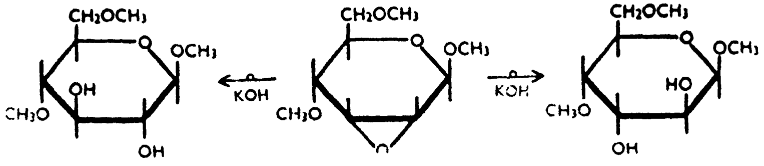


Suppose we follow the complete sequence of events in a hypothetica case set out below:



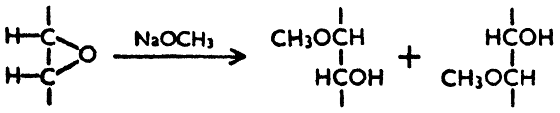
(A) (B) (C) (D)

(C) is clearly of the same configuration as (A), but in (D) there have been in effect two Walden inversions. Thus 2,3-anhydro 4,6-dimethyl β-methylalloside with potassium hydroxide gives 4,6-dimethyl β-methylglucoside and 4,6-dimethyl β-methylaltroside.*



Glucoside Alloside Altroside

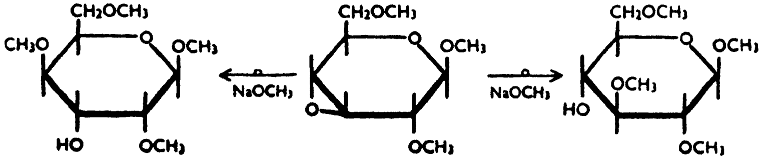
When sodium methoxide is used as a hydrolysing agent, the course of the reaction can be seen more clearly, for the methoxyl anion (OCH_3^-) which enters the system can attack either of the two carbon centres, and at the point of entry of the methoxyl inversion takes



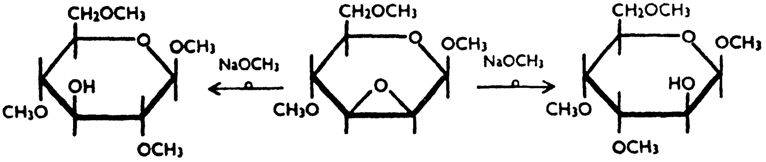
---

* Peat and Wiggins, *J.C.S.*, 1938, 1810.

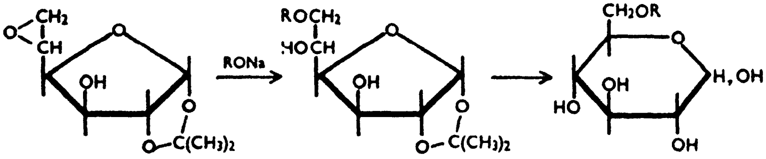
place. To take definite examples, 2,6-dimethyl 3,4-anhydro β-methylalloside gives, with sodium methoxide a mixture of 2,3,6-trimethyl β-methylglucoside and 2,4,6-trimethyl β-methylguloside ;* and 2,3-anhydro 4,6-dimethyl β-methylmannoside gives approximately equal amounts of the corresponding 2,4,6-trimethyl β-methylglucoside and 3,4,6-trimethyl β-methylaltroside.† It should be noted, however, that it is not invariably possible to isolate both products, since the proportions vary, that is the chances of the two oxygen bridges being split are not equal in all such compounds owing to steric factors. An extreme example of this is seen when one of the oxygen bridges is attached to a carbon atom which is not asymmetric as in 1,2-monoacetone 5,6-anhydro glucofuranose, where fission with sodium alkoxides followed by hydrolysis of the acetone residue produces exclusively 6-alkyl glucoses, a large number of which have been prepared by Ohle and his co-workers,‡ as well as 6-phenyl-, 6-phthalimido-, 6-diphenylamino-, 6-N-alanino-glucose and other derivatives.



Guloside Alloside Glucoside



Glucoside Mannoside Altroside



---

* Peat and Wiggins, *J.C.S.*, 1938, 1088.

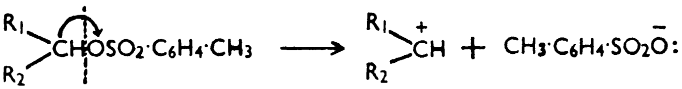
† Lake and Peat, *J.C.S.*, 1938, 1417.

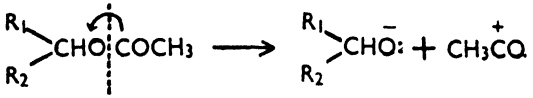
‡ *Ber.*, 1938, 71, 1843.

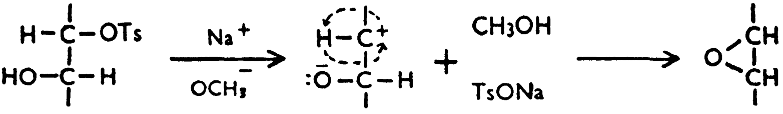
The use of such reactions, only a small number of which have been detailed, in the preparation of substituted sugars and for interconversion reactions is obvious. Before going on to consider some applications to the amino sugars, let us turn aside to consider the possible reaction mechanisms involved in what may, at first sight, seem to be mysterious changes.

Formation of the Ethylene Oxide Ring

When a toluene sulphonic ester or a halide is hydrolysed the fission occurs as shown, the arrow indicating the direction of the electron shift, quite unlike the case of a carboxylic ester. This process results in the incipient production of a carbonium cation in the former case. The adjacent *trans* hydroxyl group loses a proton, and becomes an anion, which is on the side opposite to the positive charge, so that when the charges are neutralised the hydrogen atom on the carbonium cation is displaced to the other side and the oxide ring is formed with Walden inversion on the carbon atom which originally carried the tosyl group.

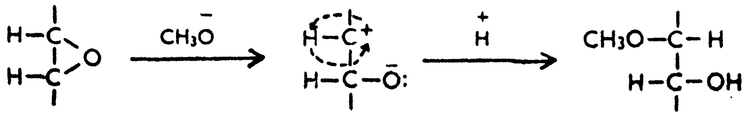






Fission of Ethylene Oxide Rings

The active agent in this case is the methoxyl anion CH_3O^-, and the reaction is not intramolecular as above but intermolecular. The first step is for a carbonium cation to be produced at one of the carbon centres, and the oxygen atom acquires a negative charge. The



methoxyl can then approach the carbonium cation from the side opposite to that originally occupied by a link to the oxygen atom, and this results in inversion of configuration on the carbon atom acquiring the methoxyl group.

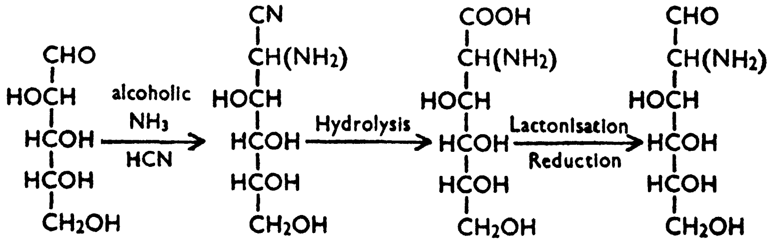
The Amino Sugars

The chief interest in this group of compounds arises from the fact that two hexosamines,[*] chitosamine (D-glucosamine) and chondrosamine (D-galactosamine) are found as building stones in natural products, the former in the amino polysaccharide, chitin, the skeletal polysaccharide of crustacea and fungi, in mucoproteins and mucopolysaccharides, the latter in the chondroitin sulphuric acid of cartilage and tendons.

D-*Glucosamine* (Chitosamine)

The best source of this substance is chitin, and owing to the fact that until recently its precise constitution was unknown, the name chitosamine has been generally applied to it. In chitin this hexosamine, which is combined in a chain structure, has the amino group acetylated, as is so in many natural products such as hyaluronic acid sulphate (from the cornea of the eye) and in the mucopolysaccharide of egg white known as ovomucoid.

Hydrolysis of chitin with hydrochloric acid gives chitosamine hydrochloride, two isomeric α- and β-forms exhibiting mutarotation being known. The relationship of this substance to glucose (or mannose) is established by its conversion to glucosazone and by its synthesis from D-arabinose,[†] hence it is clearly either 2-amino-D-glucose or 2-amino-D-mannose. When attempts were made to re-



D-Arabinose

Chitosaminic acid

Chitosamine

---

[*] See Levene, *Hexosamines and Mucoproteins*, Longman's, 1926.

[†] Fischer and Leuchs, *Ber.*, 1902, **35**, 3787; 1903, **36**, 24.

move the amino group with nitrous acid, an anhydro sugar known as chitose containing a 2,5-anhydro ring was obtained. In an attempt to circumvent this behaviour Irvine and Hynd* prepared benzylidene methylglucosaminide, and this substance gave a benzylidene mannose on treatment with nitrous acid, D-mannose being obtained on hydrolysis. By another series of complex reactions, however, involving treatment of dimethylaminomethylglucosaminide with barium hydroxide, glucose had been isolated. Obviously therefore at some stage a Walden inversion had taken place, although the general view has prevailed that chitosamine is 2-amino-D-glucose.†

This has been definitely confirmed by the work of Haworth and Peat,‡ utilising ethylene oxide ring compounds (page 76). When 2,3-anhydro 4,6-dimethyl β-methyl-D-mannoside (I) is treated with sodium methoxide the products obtained, as mentioned previously (page 72) are the 2,4,6-trimethyl β-methylglucoside (II) and the 3,4,6-trimethyl β-methylaltroside (III). If ammonia is used instead of sodium methoxide the products to be expected are 2-amino 4,6-dimethyl β-methyl-D-glucopyranoside (IV) and 3-amino 4,6-dimethyl β-methyl-D-altropyranoside (V). Experimentally two different methylated amino sugars were isolated and were separated by fractional crystallisation as the N-acetates. It was possible to decide which derivative had the amino group on C_2 since one of the products on methylation was shown to give a derivative of 3-amino altrose identical with that prepared from "epi"-glucosamine (see page 78) and the other on methylation a 2-amino 3,4,6-trimethyl β-methyl-D-glucopyranoside, isolated as the N-acetyl derivative (VI) and identical with a product prepared by the methylation of natural chitosamine (VII) in which of course the amino group is known to be on C_2. This established by chemical means that chitosamine was really 2-amino-D-glucose and was not a derivative of mannose, a conclusion which was confirmed independently by X-ray methods using the isomorphous glucosamine hydrochloride and hydrobromide without making any previous assumptions.§

It is of interest that N-methyl-L-glucosamine|| is a constituent of the important antibiotic streptomycin.

---

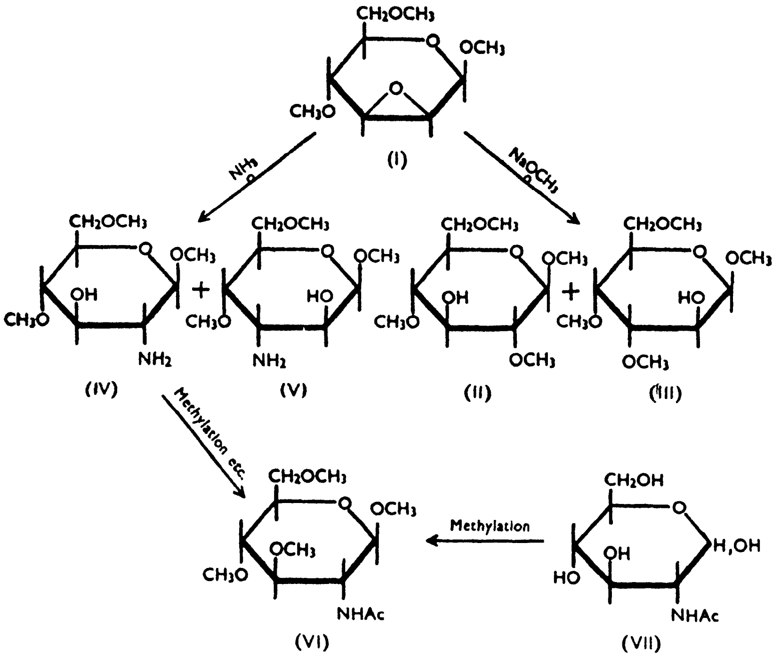
* *J.C.S.*, 1912, 1128; 1914, 698.

† Karrer and Mayer, *Helv. Chim. Acta*, 1937, **20**, 407.

‡ Cutler, Haworth and Peat, *J.C.S.*, 1937, 1979; Haworth, Lake and Peat, *ibid.*, 1939, 271.

§ Cox and Jeffrey, *Nature*, 1939, **143**, 894.

|| Kuehl, Flynn, Holly, Mozingo and Folkers, *J. Amer. Chem. Soc.*, 1947, **69**, 3032.



2-Acetamido 3,4,6-trimethyl
β-methylglucopyranoside
[N-Acetyl trimethyl β-
methylglucosaminide]

N-Acetyl glucosamine

Chondrosamine

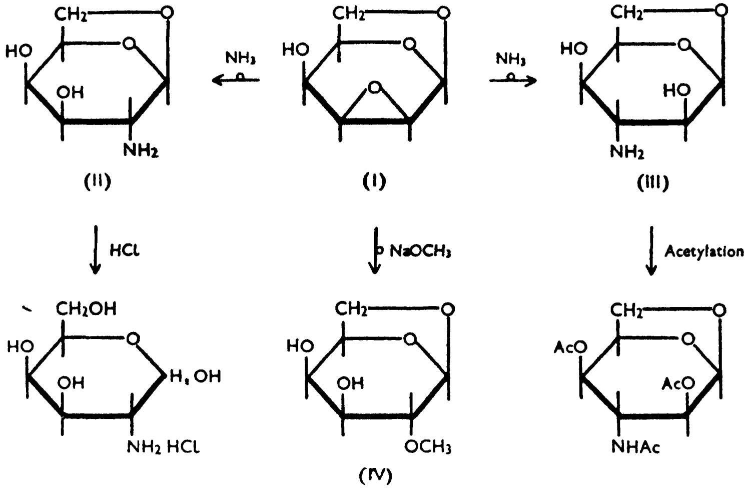
This important hexosamine bears the same relationship to galactose as chitosamine to glucose. It is obtained by the hydrolysis of chondroitin sulphuric acid derived from cartilage or nasal septa; treatment with phenylhydrazine gives D-galactose phenylosazone and the choice of structure in this case therefore lies between 2-amino-D-galactose and 2-amino-D-talose. This question has been settled in favour of the galactose derivative,* again making use of anhydro sugars in a constitutional synthesis. The starting material was 2,3-1,6-dianhydro-β-D-talose (I), a compound prepared from β-galactosan as follows:

β-Galactosan → 3,4-monoacetone 1,6-anhydrogalactose → 2- methanesulphonyl 1,6-anhydrogalactose → 2,3-1,6-dianhydro β-D-talose.

Treatment with ammonia gave a mixture of products subsequently identified as 2-amino 1,6-anhydro β-D-galactose (II) isolated as the hydrochloride and a smaller quantity of 3-amino 1,6-anhydro β-D-idose (III) (isolated as a crystalline acetamido diacetate, m.p. 245-6°). When (I) was treated with sodium methoxide 2-methyl 1,6-anhydro β-D-galactose was obtained in 60% yield. In order to decide between

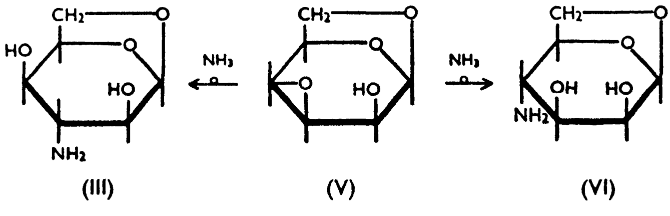
---

* **James, Smith, Stacey and Wiggins, *J.C.S.*, 1946, 625.**



Chondrosamine
hydrochloride

the structure of the product giving the crystalline hydrochloride and the acetamido diacetate, m.p. 245°, the action of ammonia on 3,4-1,6-dianhydro β-D-talose (V) was studied. Two products (III) and (VI) were obtained, one of which gave the same crystalline acetamido

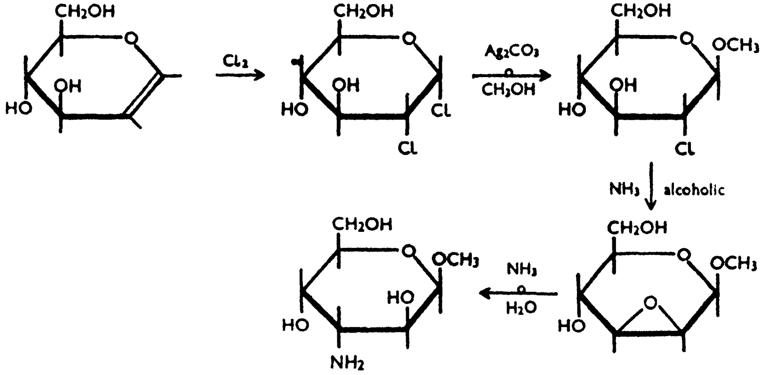


diacetate, m.p. 245°, as that isolated in the first series of reactions. Examination of the formulae shows that this could only have been the D-idose derivative (III), since this is the only product which could be common to both series of reactions. It followed therefore that the product which gave the crystalline hydrochloride from the first experiments must have been the D-galactose derivative. When this material was treated with acid to break the glycosan ring, 2-amino-

D-galactose hydrochloride was obtained which proved to be identical with chondrosamine hydrochloride and gave the same crystalline β-pentaacetyl derivative and an identical X-ray powder photograph.

"Epi"-glucosamine. 3-Amino-Altrose

By acting upon glucal (page 63) with chlorine followed by glycoside formation, treatment with ammonia and hydrolysis, Fischer* hoped to prepare the epimer of glucosamine. The product however formed an osazone still containing an amino group and was in fact a 3-aminohexose. The probable course of the reaction is shown below, the final product being a 3-amino methylaltrosaminide.



The same product was also obtained from 2-tosyl 3,4,6-triacetyl β-methylglucoside by treatment with ammonia† 2,3-anhydro β-methylmannoside being presumably formed as the intermediate compound.

6-*Amino Glucose*. Fischer and Zach‡ treated 6-bromo triacetyl methylglucoside with ammonia; hydrolysis gave 6-amino glucose.

1-*Amino Fructose* (*Fructosamine*). By the reduction of glucosazone with zinc dust and acetic acid in alcohol§ or, better by catalytic hydrogenation,|| 1-aminofructose is obtained. With phenylhydrazine it is converted to glucosazone and with nitrous acid gives fructose, thus giving another method for the conversion of glucose into fructose.

---

* Fischer, *Ber.*, 1914, **47**, 196.

† Bodycote, Haworth and Hirst, *J.C.S.*, 1934, 151.

‡ *Ber.*, 1912, **45**, 456.

§ Fischer, *Ber.*, 1886, **19**, 1920.

|| Maurer and Schiedt, *Ber.*, 1935, **68**, 2187.

CHAPTER V

OLIGOSACCHARIDES

The Disaccharides

As the name indicates, members of the disaccharide group are made up of two monosaccharide units. These may be alike, as in maltose, which on hydrolysis gives two molecules of glucose, or different, as in sucrose, a disaccharide composed of a fructose unit joined to a glucose residue. Most common disaccharides are dihexoses with the molecular formula $C_{12}H_{22}O_{11}$ derived from two $C_6H_{12}O_6$ molecules by the loss of the elements of water, although a few natural members of the group such as primeverose are known in which a pentose and a hexose are united together, and by degradation methods disaccharides containing tetrose residues have been prepared.

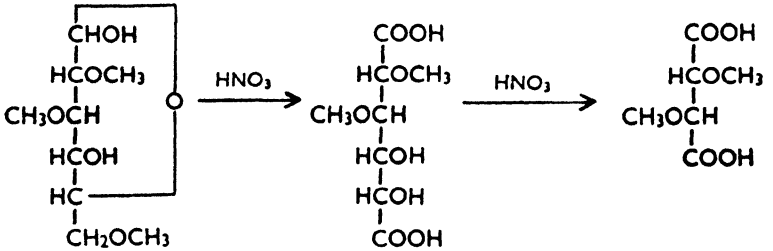
All natural disaccharides are glycosides, and at least one of the sugar units is joined to the other through the so-called reducing group just as methanol is linked to glucose in the methylglucosides. Since there are five free hydroxyl groups in a hexose the possibilities of combination are numerous, especially when it is remembered that the combining sugars can be joined by either α- or β-linkage.

For convenience, the disaccharides are often classified as reducing and non-reducing. In the former case Fehling's solution but not Barfoed's reagent (copper acetate in acetic acid) is reduced—a useful distinction in a preliminary analysis. On the one hand reducing disaccharides, such as maltose and lactose, form osazones and can be crystallised in the isomeric α- and β-forms which mutarotate in solution. It is clear, therefore, that the reducing group of one of the sugar components is free. That both are not free is seen from many facts, such as the composition of the disaccharide osazones, maltosazone for example, having the formula $C_{24}H_{32}O_9N_4$, and not $C_{36}H_{42}O_7N_8$, so that two but not four phenylhydrazino residues are present. Since the hydroxyl group on C_2 is used in osazone formation, the bridge from the reducing group of the one hexose could be only to C_3, C_4, C_5 or C_6 of the other hexose. On the other hand, in a non-reducing disaccharide which can neither mutarotate nor form an osazone, it is obvious that both the reducing groups of the constituent sugars are glycosidically linked to one another; examples of this type of structure are furnished by sucrose and trehalose.

Reducing Disaccharides with 1,4-Linkages

Maltose. The well-known disaccharide maltose is an intermediate in the process of brewing, being produced from starch by the action of barley diastase, in a yield of about 80%. A reducing sugar, it exists in both α- and β-forms which show mutarotation in aqueous solution; hydrolysis by heating with mineral acid or with maltase gives two molecules of D-glucose. The specific rotation of the β-form $[\alpha]_D + 112° \rightarrow + 130°$ (equilibrium), and its hydrolysis by maltase (α-glucosidase) indicates the internal glycosidic link to be α.

Maltose (I) on methylation gave a heptamethyl methylmaltoside* (II) which on hydrolysis yielded 2,3,4,6-tetramethyl glucose (III) and a trimethyl glucose which was subsequently shown to be 2,3,6-trimethyl glucose (IV) since by oxidation with nitric acid it could be converted into a dimethyl saccharic acid, and on more vigorous oxidation into L(+)-dimethoxysuccinic acid. From this result it is clear that the non-reducing residue which is a glucopyranose unit, since it gives 2,3,4,6-tetramethyl glucose, must be linked to the second (reducing) glucose unit through either C_4 or C_5. A decision between these two possibilities was reached† by oxidising maltose with bromine water to give maltobionic acid, thereby breaking the oxide ring in the reducing portion of the molecule. Complete methylation gave a methyl octamethyl maltobionic ester (V), hydrolysis of

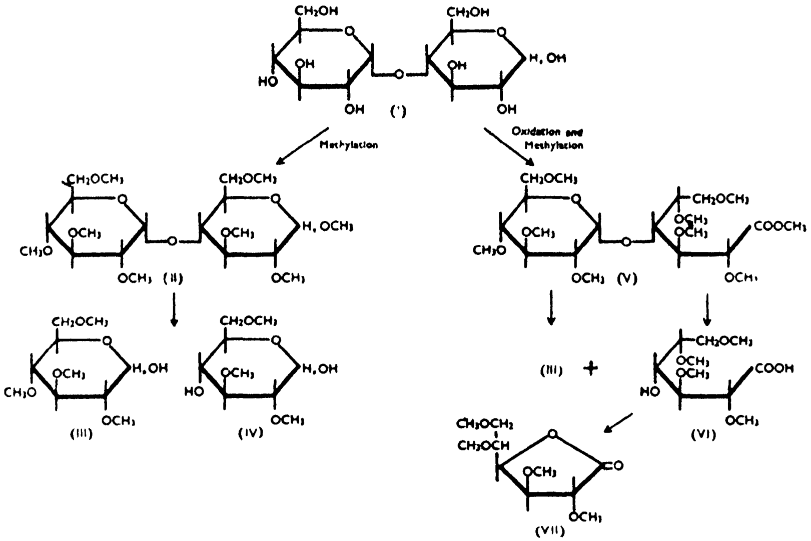


2,3,6-Trimethyl glucose 2,3-Dimethyl saccharic acid L(+)-Dimethoxysuccinic acid which gave crystalline, 2,3,4,6-tetramethyl glucose (III), and 2,3,5,6-tetramethyl gluconic acid (VI) which was readily converted into the γ- or furano-lactone (VII). This experiment proved that the bridge between the glucose units connected C_1 and C_4 and that both rings were pyranose. Maltose must therefore be designated as 4-[α-D-glucopyranosido]-D-glucopyranose. The results of two other methods which support this conclusion may be cited here.

---

* Haworth and Leitch, *J.C.S.*, 1919, 809.

† Haworth and Peat, *J.C.S.*, 1926, 3094.

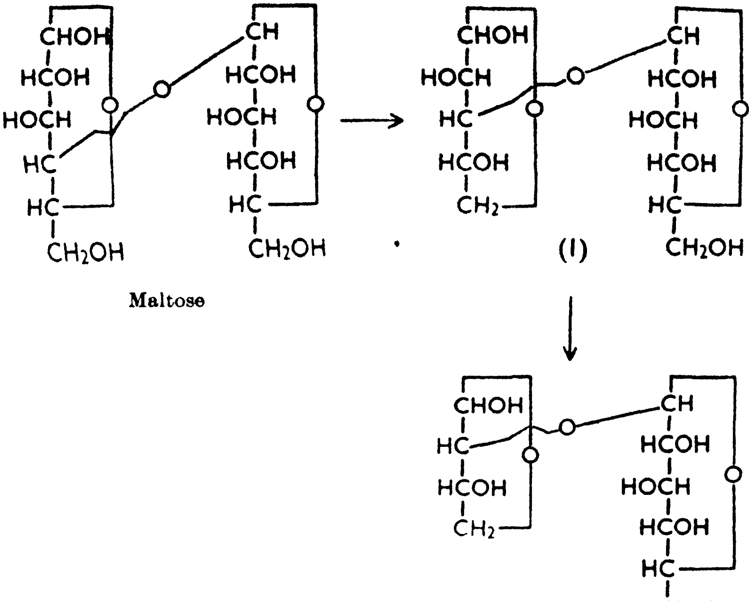


Levene and Sobotka* studied the lactonisation of maltobionic acid and concluded because of its rapid formation and hydrolysis that a δ-lactone was formed exclusively, indicating that the hydroxyl group on C_4 was substituted. Zemplén† carried out experiments to determine how far degradation had to proceed before the truncated disaccharide portion no longer formed an osazone. Thus maltose oxime was degraded by a modification of Wohl's method (see page 17) to give first [α-D-glucosido]-D-arabinose (I), and then by repeating the process [α-D-glucosido]-D-erythrose (II). The degradation could not be continued beyond this point and the new disaccharide would not form an osazone. Zemplén concluded that the next hydroxyl group was occupied by the glycosidic bridge, which must therefore have involved C_4 in the original maltose molecule.

---

* *J. Biol. Chem.*, 1927, 71, 471.

† *Ber.*, 1926, 59, 1254; 1927, 60, 1555.



Maltose

(I)

(II)

2-[α-D-Glucosido]-D-erythrose

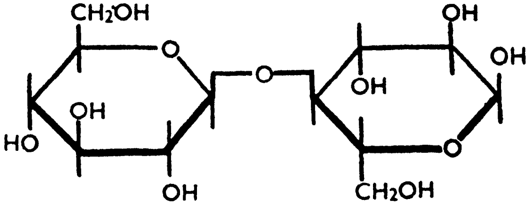
Cellobiose. By subjecting cellulose to acetolysis, a process of simultaneous hydrolysis and acetylation with a mixture of acetic anhydride and sulphuric acid, cellobiose octaacetate is obtained. The constitution of the reducing disaccharide, cellobiose, obtained from this product on deacetylation, is of primary importance owing to its origin, although cellobiose itself is not found free in nature. [The neat method of deacetylation introduced by Zemplén* may be mentioned here. Instead of using an excess of caustic alkali to saponify the acetyl groups, a small quantity of sodium dissolved in methanol is employed. The octaacetyl cellobiose is dissolved in chloroform and a solution of sodium in methanol containing about one twentieth of the theoretical amount of sodium is added. Smooth deacetylation takes place with the production of methyl acetate due to the catalytic action of sodium methoxide. The substitution of potassium for sodium and subsequent treatment with perchloric acid is a recent improvement in the method† since the contamination of the deacetylated product by sodium acetate is thereby avoided.] β-Cellobiose ($[\alpha]_D$ + 14° $\longrightarrow$ + 35° in water) has the low specific rotation typical of β-D-glycosides, and is hydrolysed by emulsin to give two molecules of glucose in agreement with this assignment of configuration.

---

* *Ber.*, 1926, **59**, 1254; 1927, **60**, 1555.

† Bonner and Koehler, *J. Amer. Chem. Soc.*, 1948, **70**, 314.

The proof of the structure of cellobiose follows parallel lines to that already outlined for maltose. Heptamethyl methylcellobioside* prepared by methylation gave on hydrolysis 2,3,6-trimethyl- and 2,3,4,6-tetramethyl- glucose, and the hydrolysis products of methyl octamethyl cellobionate† were the same as those given by the corresponding maltobionic ester, namely tetramethyl glucopyranose and 2,3,5,6-tetramethyl gluconic acid which was identified as the corresponding crystalline γ-lactone. Cellobiose and maltose are therefore identical in structure *except for* the direction of the disaccharide bridge which as already proved is β in cellobiose and the sugar is 4-[β-D-glucopyranosido]-D-glucopyranose. The same conclusion about the 1,4-bridge was also reached by Levene and Zemplén using the methods outlined for maltose. Cellobiose has been synthesised by methods which leave no doubt of its constitution, as will be seen later in this chapter.



Cellobiose (α-form)

Lactose. Lactose is the reducing disaccharide found in the milk of mammals, and on hydrolysis gives both glucose and galactose. Fischer‡ showed that the galactose was contained in the non-reducing portion since lactosazone on conversion to the osone followed by hydrolysis with acid gave galactose and glucosone. Like cellobiose, lactose belongs to the β-series on account of its specific rotation ($[\alpha]_D + 35° \longrightarrow + 55°$) and because it is only hydrolysed by β-galactosidases. Methylation and hydrolysis of heptamethyl methyllactoside gave 2,3,6-trimethyl glucose and 2,3,4,6-tetramethyl galactose.§ The lactobionic acid produced on oxidation, after complete methylation and hydrolysis* gave tetramethyl galactopyranose and 2,3,5,6-tetramethyl gluconic acid, showing that the bridge link from the galactose to the glucose unit must be to C_4. Lactose thus

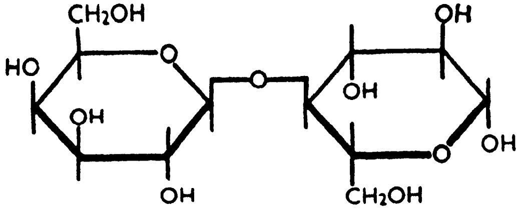
---

* Haworth and Hirst, *J.C.S.*, 1921, 193.

† Haworth, Long and Plant, *J.C.S.*, 1927, 2809.

‡ *Ber.*, 1888, **21**, 2633.

§ Haworth and Leitch, *J.C.S.*, 1918, 188.



Lactose (β-form)

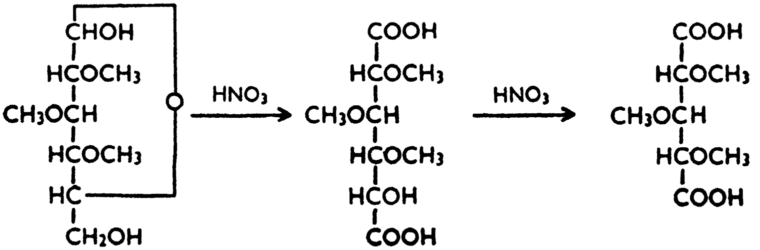
falls into the same group as maltose and cellobiose, and is 4-[β-D-galactopyranosido]-D-glucopyranose; the only difference between the formulae of lactose and cellobiose being the inversion of H and OH on C_4 in the non-reducing residue. The above constitutional proof is supported by the results obtained by Levene from the lactone of lactobionic acid, the degradation of lactose to 2-[β-D-galactosido]-D-erythrose, by Zemplén and by its synthesis (page 96).

Although the 1,4-disaccharide linkage is present in these three well-known disaccharides it is by no means universal.

Reducing Disaccharides with 1,6-Linkages

Gentiobiose. The action of invertase on a trisaccharide called gentianose, isolated from the root of the yellow gentian, gives a reducing disaccharide, gentiobiose, which is also present in the natural glycoside, amygdalin (page 180).

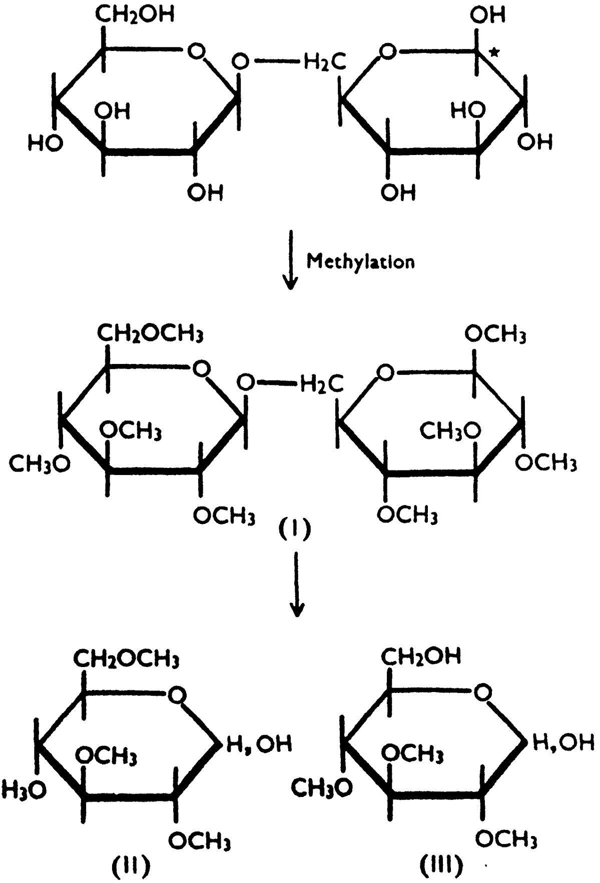
Two molecules of glucose are produced from gentiobiose on hydrolysis with acids or with emulsin, obviously therefore it is a β-glycoside which is indeed to be expected from its low equilibrium rotation in



2,3,4-Trimethyl glucose   Trimethyl saccharic acid   i-*Xylo*trimethoxyglutaric acid water ([α]$_D$ +9·6°). The mode of union of the two glucose residues was established by methylation studies.* Heptamethyl methylgentiobioside (I) on hydrolysis gave 2,3,4,6-tetramethyl glucopyranose (II) and a liquid trimethyl glucose (III) which gave a crystalline β-methylglucoside. This fragment was proved to be 2,3,4-trimethyl glucose; oxidation gave a trimethyl saccharic acid proving the absence of a methyl residue on C_6, more vigorous oxidation gave *i-xylo*-trimethoxyglutaric acid. The subsequent synthesis of 2,3,4-trimethyl β-methylglucoside (page 52) confirmed the above conclusions. The isolation of 2,3,4-trimethyl glucose from gentiobiose suggests that

---

* Haworth and Long, *J.C.S.*, 1927, 544.



---

* Haworth and Wylam, *J.C.S.*, 1923, 3120.

the most probable formulation would comprise two glucopyranose units, linked through C_1 in one unit to C_6 in the other through a β-glucosidic link, and gentiobiose is indeed recognised to be 6[β-D-glucopyranosido]-D-glucopyranose.

It will be seen later how the above constitution has been confirmed by synthesis.

Melibiose. The second disaccharide of this class, melibiose, occurs in combination with fructose in the trisaccharide, raffinose, which yields melibiose on treatment with invertase. Like gentiobiose, melibiose is reducing, mutarotates, and forms an osazone. It is isomeric with lactose in the sense that it gives glucose and galactose on hydrolysis. The galactose portion in this case also is found in the non-reducing moiety, since heptamethyl methylmelibioside* on hydrolysis gave tetramethyl galactopyranose and the same 2,3,4-trimethyl glucose that methylated gentiobiose gave on hydrolysis. The methyl ester of octamethyl melibionic acid gave on hydrolysis tetramethyl galactopyranose and a tetramethyl gluconic acid which would not form a lactone but readily gave tetramethyl saccharic acid (identified as its crystalline amide and methylamide) on oxidation. These results established the tetramethyl gluconic acid to be the 2,3,4,5-derivative from which it follows that the oxygen bridge must be connected to C_6 in the glucose fragment of melibiose.† Melibiose is therefore another representative disaccharide in which one sugar unit is glycosidically joined to the primary alcoholic group of another.

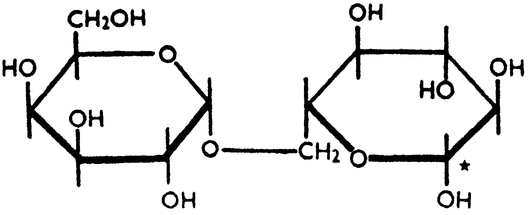
There is a second important difference between melibiose and gentiobiose, apart from the galactose component of the former, in that the glycosidic linkage is α and not β. This may be deduced from the high specific rotation of the sugar ($[\alpha]_D = 113°$ for the β-form) and from a comparison of the specific rotation of the methyl octamethyl melibionate with corresponding derivatives from other disaccharides. Ample confirmation was secured by its synthesis and that of 6-[β-D-galactopyranosido]-D-glucopyranose which was found to have properties markedly different from those of melibiose (see page 97).

Melibiose is therefore 6-[α-D-galactopyranosido]-D-glucopyranose, the β-form of which is shown on next page.

---

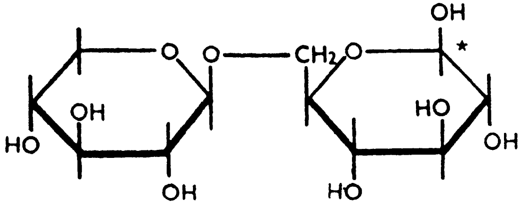
* Haworth, Hirst and Ruell, *J.C.S.*, 1923, 3125.

† Haworth, Loach and Long, *J.C.S.*, 1927, 3146.



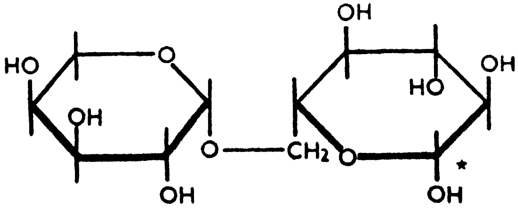
Melibiose (β-form)

Primeverose, Vicianose and Rutinose. Three well-defined disaccharides which are also 6-substituted glucose derivatives, have been isolated from certain natural glycosides. Primeverose, the sugar residue in the glycoside of madder root, ruberythric acid,* and found also in plants of the genus *Primula*, contains a D-xylose residue and synthesis has established it to be 6-[β-D-xylopyranosido]-D-glucopyranose.†



Primeverose (β-form)

Vicianose which is found in the cyanogenetic glycoside from the seeds of *Vicia angustifolia* is an L-arabinoside, shown to be 6-[β-L-arabopyranosido]-D-glucopyranose.



Vicianose (β-form)

---

* Richter, *J.C.S.*, 1936, 1701.

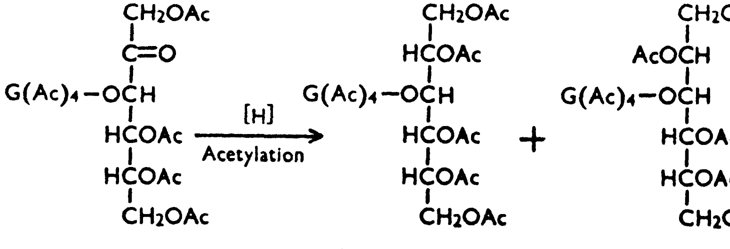
† Helferich and Bredereck, *Annalen*, 1928, **465**, 166; McCloskey and Coleman, *J. Amer. Chem. Soc.*, 1943, **65**, 1778.

Rutinose from the glycosides rutin and hesperidin has also been synthesised (as the heptaacetate)[*] and is 6-[β-L-rhamnosido]-D-glucopyranose.

Reducing Disaccharides with 1,3-Linkages

Turanose. A trisaccharide, melezitose, is found as an exudation on certain trees such as the Douglas fir, larch, lime, and poplar, from which on hydrolysis with acid and treatment with yeast a reducing disaccharide, turanose, may be isolated. The history of the evolution of the formula of this sugar as 3-[α-D-glucopyranosido]-D-fructose is somewhat confusing,[†] but a conclusive proof due to Hudson has been furnished, based on the formula we have already deduced for maltose.

By the catalytic hydrogenation of the *keto* form of turanose octaacetate, E. Pacsu and his co-workers[‡] had isolated, after reacetylation to substitute the newly introduced hydroxyl group, 3-[α-D-glucopyranosido]-D-sorbitol nonaacetate and 3-[α-D-glucopyranosido]-D-mannitol nonaacetate, m.p. 142°, the above compounds being so named, of course, on the assumption that turanose was 3-[α-D-glucopyranosido]-D-fructose. Octaacetyl maltose had previously been converted by way of acetobromomaltose and maltal into 4-[α-D-glucosido]-D-mannose[§] by the following steps:



3-[Glucopyranosido]-D-sorbitol nonaacetate   3-[Glucopyranosido]-D-mannitol nonaacetate

G = Glucosyl residue $C_6H_{11}O_5$ G(Ac)$_4$ = Tetraacetyl glucosyl

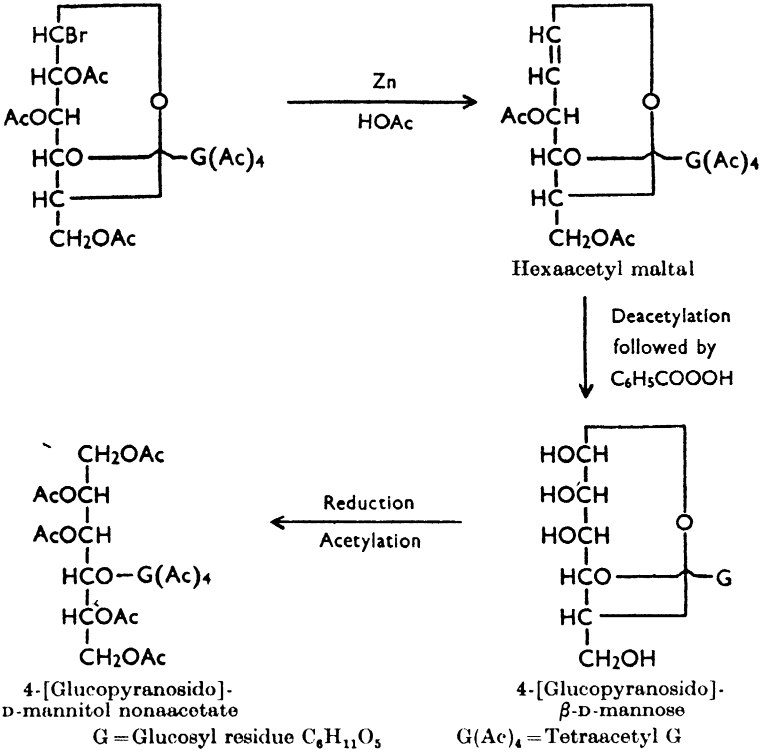
---

[*] Zemplén and Gerecs, *Ber.*, 1934, **67**, 2049.

[†] See *Advances in Carbohydrate Chemistry*, 1946, **2**, 1.

[‡] *J. Amer. Chem. Soc.*, 1939, **61**, 2675.

[§] Haworth, Hirst and Reynolds, *J.C.S.*, 1934, 302.



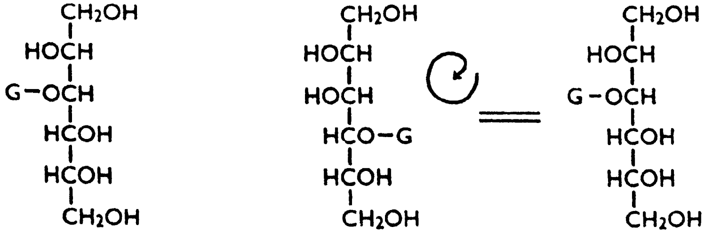
Hexaacetyl maltal

4-[Glucopyranosido]-D-mannitol nonaacetate

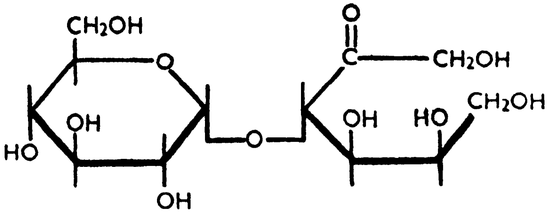
4-[Glucopyranosido]-β-D-mannose

G = Glucosyl residue $C_6H_{11}O_5$ G(Ac)$_4$ = Tetraacetyl G

Hudson accordingly prepared 4-[α-D-glucosido]-D-mannose by this route, reduced it to the corresponding 4-mannitol derivative and found that the nonaacetate (m.p. 142°) produced on acetylation was identical with the compound produced from keto-turanose octa-acetate by Pacsu. An inspection of the formulae for 3 and 4 substituted mannitol derivatives will show that owing to the special



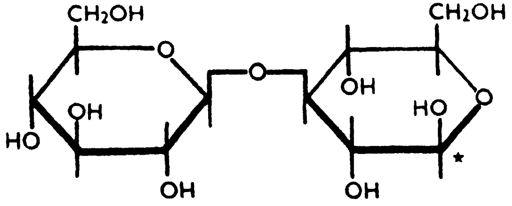
symmetry of the molecule the compounds are identical. The formula of turanose is therefore proved, at any rate with respect to its linkage, since the only other possible structure from this evidence, namely, 4-[α-D-glucopyranosido]-D-fructose is negatived by the fact that turanosazone is not identical with maltosazone.



Turanose (Keto-form)

Pacsu isolated five crystalline turanose octaacetates, two forms with the fructose unit furanose, two with it pyranose and one keto form already mentioned; there is no direct evidence at present as to the ring form of the fructose residue in turanose itself, although combined in melezitose it exists as a furanose form.

Laminaribiose. V. C. Barry,* by the partial hydrolysis of the polysaccharide laminarin extracted from seaweeds, isolated a reducing diglucose, laminaribiose, in which the glucose residues are joined by 1,3-β-links.



Laminaribiose (β-form)

Sophorose. A reducing disaccharide obtained† by the hydrolysis of a glycoside isolated from *Sophora japonica* has been found to be identical with the synthetic 2-[β-D-glucopyranosido]-D-glucose prepared by K. Freudenberg and his co-workers.

Non-Reducing Disaccharides

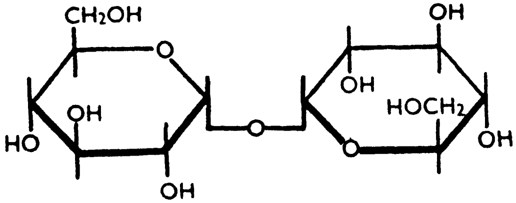
Trehalose. Fungi, yeasts and certain plants contain a non-reducing disaccharide, which also appears in "trehala manna". This substance, trehalose, is relatively resistant to hydrolysis with mineral acid, an indication of its pyranose structure, the only identifiable product of the hydrolysis being D-glucose. Assuming, for the moment, a pyranose structure for both the glucose fragments, there are three possible ways of uniting them, namely by *αα*, *ββ* or *αβ* linkages. Since trehalose has a very high specific rotation, $[\alpha]_D + 197°$, the first constitution would seem the most probable, and this has been confirmed by the synthesis of the other two possibilities (see page 97).

---

* *Sci. Proc. Roy. Dublin. Soc.*, 1941, **22**, 423.

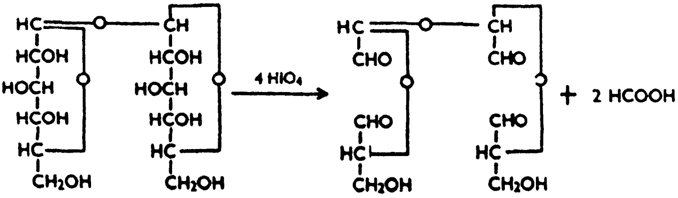
† Rabaté, *Bull. Soc. Chim.*, 1940, [5] 7, 565; *Naturwiss*, 1947, **34** II, 344.

By the hydrolysis of octamethyl trehalose two molecular proportions of 2,3,4,6-tetramethyl glucose were obtained* which is in agreement with the formulation of trehalose as 1-[α-D-glucopyranosido]-α-D-glucopyranoside.



Trehalose

Trehalose (I) on oxidation with periodic acid required four molecules of the oxidant, and two molecules of formic acid were liberated. Oxidation of the dialdehyde D′,D′-oxy *bis* (D-hydroxymethyldiglycollic aldehyde) (II) resulted in the isolation of a crystalline strontium salt [Distrontium D′,D′-oxy *bis* (D-hydroxymethyldiglycollate)] which on hydrolysis and further oxidation gave oxalic acid and D-glyceric acid. The only structure in harmony with these facts is the dipyranose structure.†



(I) (II)

Trehalose

Sucrose. There is perhaps no need to stress the importance of sucrose as an article of diet; what is not always realised, however, is that cane sugar, of all pure organic chemicals, is the most important in a quantitative sense, its tonnage far exceeding that of any other compound. Although the main sources are sugar cane and sugar beet, sucrose is found in all photosynthetic plants, in fact some workers have held that it is the primary product of photosynthesis.

---

* Schlubach and Maurer, *Ber.*, 1925, **58**, 1178.

† E. L. Jackson and Hudson, *J. Amer. Chem. Soc.*, 1939, **61**, 1530.

The general properties of sucrose will be familiar to most students of chemistry. It is non-reducing, but is exceedingly readily hydrolysed by very dilute acids and by invertase, to give a molecule of glucose and a molecule of fructose from each sucrose molecule. During this process of hydrolysis, inversion of the sign of the optical rotation takes place, which is due to the strong laevorotation of fructose outweighing the positive value for the equilibrium rotation of glucose. The process is generally called, therefore, the inversion

$$\underset{(+66{\cdot}4^\circ)}{C_{12}H_{22}O_{11}} + H_2O = \underset{(+52{\cdot}5^\circ)}{C_6H_{12}O_6} + \underset{(-92^\circ)}{C_6H_{12}O_6}$$

of cane sugar and since it is a unimolecular reaction catalysed by hydrogen ions, and easy to follow polarimetrically, it has been useful for the determination of the relative strengths of acids. Moreover by measurements of the rotations before and after hydrolysis, accurate estimates of the amounts of sucrose in solution may be made in the technical operations of its manufacture.

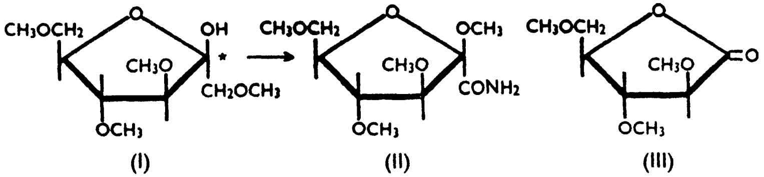
Although sucrose has not yet been synthesised by chemical means, its constitution is now well established. Octamethyl sucrose first obtained by Purdie and Irvine,* gave on hydrolysis crystalline 2,3,4,6-tetramethyl glucose, although at the time this work was carried out the position of the methyl groups was undecided.

Haworth and Law† prepared octamethyl sucrose by the sodium hydroxide methyl sulphate method and also obtained crystalline tetramethyl glucose. From the specific rotation of the mixture of tetramethyl sugars (i.e. tetramethyl glucose and a tetramethyl fructose), produced on hydrolysis, it was possible to calculate the equilibrium rotation of the tetramethyl fructose component of the mixture to be about $[\alpha]_D + 30^\circ$. Haworth pointed out that this value was markedly different from that of the crystalline tetramethyl fructopyranose or normal tetramethyl fructose as it was then called ($[\alpha]_D - 121^\circ$ at equilibrium) so that the fructose component was clearly of a constitution different from that of the tetramethyl fructose prepared from the normal methylfructosides. Because of the difficulty involved in separating two tetramethyl hexoses by vacuum distillation, Haworth* hydrolysed an incompletely methylated sucrose, heptamethyl sucrose. Two fractions were obtained on hydrolysis and distillation in a vacuum, the one with the higher boiling point proving to be a trimethyl glucose (since crystalline 2,3,4,6-tetramethyl glucose was obtained on methylation and hydrolysis) which was separated from a syrupy tetramethyl hexose of lower boiling point, having $[\alpha]_D + 32°$. The structure of this fragment was eventually established by the oxidation methods, described in Chapter II, to be tetramethyl fructofuranose (1,3,4,6-tetramethyl fructose) (I),† involving the isolation of the 2,3,4,6-tetramethyl 2-ketogluconamide (II) (page 38) and trimethyl γ-arabonolactone (III). The above facts can only be explained by representing sucrose

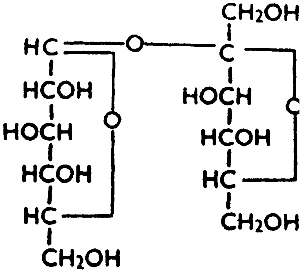
---

* Purdie and Irvine, *J.C.S.*, 1903, 1036; 1905, 1028.

† *J.C.S.*, 1916, 1314.



as a glucopyranosido-fructofuranoside which on hydrolysis yields D-glucopyranose and D-fructopyranose, the fructofuranose ring occurring in the disaccharide being unstable unless it is either anchored



Sucrose

in a glycoside, as in this case, or if the hydroxyl group on C_6 is protected as, for example, by methylation. The above representation is incomplete, however, since the configurations of the glycosidic links have not been taken into account. There are four possible ways of uniting the two components of the disaccharide:

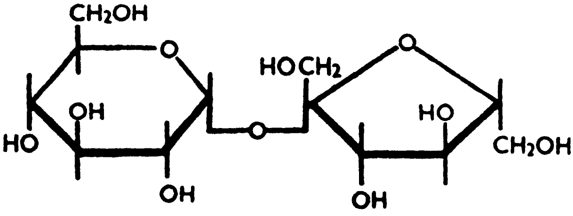
---

* *J.C.S.*, 1920, 199.

† Haworth, Hirst and Nicholson, *J.C.S.*, 1927, 1513; Haworth and Westgarth, *J.C.S.*, 1926, 880.

α-glucopyranosido-α-fructofuranoside
α-glucopyranosido-β-fructofuranoside
β-glucopyranosido-α-fructofuranoside
β-glucopyranosido-β-fructofuranoside

An examination of the mutarotation which takes place under the influence of maltase indicates that the glucose which is liberated mutarotates in a downward direction, this component is therefore to be regarded as linked as an α-glucoside;* the mutarotation of the fructose component, however, appears to be practically instantaneous. There is evidence however that a taka-invertase which hydrolyses sucrose is a β-fructofuranosidase since it hydrolyses β-methylfructofuranosides,† and from this and from calculations of the optical rotation of the fructose fragment it is highly probable that sucrose is α-D-glucopyranosido-β-D-fructofuranoside.



Sucrose

The above formulation is supported by the results of the X-ray examination of the isomorphous sodium chloride and sodium bromide addition compounds of sucrose.‡

The Synthesis of Disaccharides

Although the constitutions of the disaccharides we have been discussing have in most cases been fully established by the methods outlined, corroboration by synthetic experiments has been obtained in many cases.

---

[*] Armstrong, *J.C.S.*, 1903, 1305; Hudson, *J. Amer. Chem. Soc.*, 1909, **31**, 655.

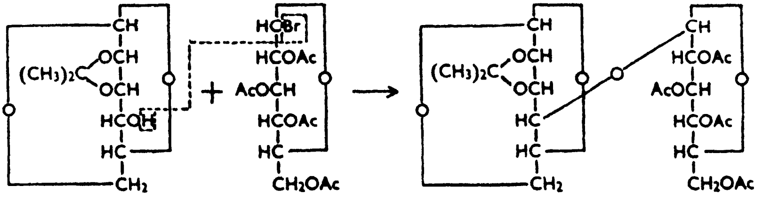
[†] Schlubach and Rauchalles, *Ber.*, 1925, **58**, 1842.

[‡] Beevers and Cochrane, *Proc. Roy. Soc.*, A, 1947, **190**, 257.

Cellobiose

Freudenberg and Nagai* obtained a small yield of cellobiose octaacetate by condensing β-glucosan (1,6-anhydro β-glucopyranose) with acetobromoglucose. A synthesis which is also a constitutional proof has been achieved by treating molten 1,2,3,6-tetraacetyl β-D-glucopyranose with dry sodium in a nitrogen atmosphere to give the 4-sodio derivative. This was condensed with acetobromoglucose in the fused state, the products acetylated and β-cellobiose octaacetate (10% yield) was isolated after chromatographic separation (see page 167) on a silica column.†

Previously another constitutional synthesis had been carried out by Hudson and his co-workers‡ by an ingenious process. β-D-Mannosan (1,6-anhydro β-D-mannopyranose, see page 66) was converted into the 2,3-monoacetone derivative (I), the only free hydroxyl group in this compound being located on C_4. Condensation with acetobromoglucose in the presence of silver carbonate gave 1,6-anhydro 2,3-monoacetone 4-[β-D-tetraacetyl glucopyranosido]-D-mannose (II). Treatment with acetic acid removed the acetone residue without affecting the biose link, and acetylation with acetic anhydride containing sulphuric acid caused the glycosan ring to open and produced 4-[β-D-glucopyranosido]-D-mannose octaacetate or *epi*-cellobiose octaacetate. The *epi*-cellobiose octaacetate was converted into the acetobromo derivative which was reduced by zinc dust and acetic acid to give the glycal (hexaacetyl cellobial); perbenzoic acid converted the glycal into the hexaacetyl cellobiose derivative (see page 63 for the corresponding transformation of glucal into mannose); acetylation gave octaacetyl cellobiose from which the disaccharide itself was obtained on deacetylation with barium methoxide (see page 96).



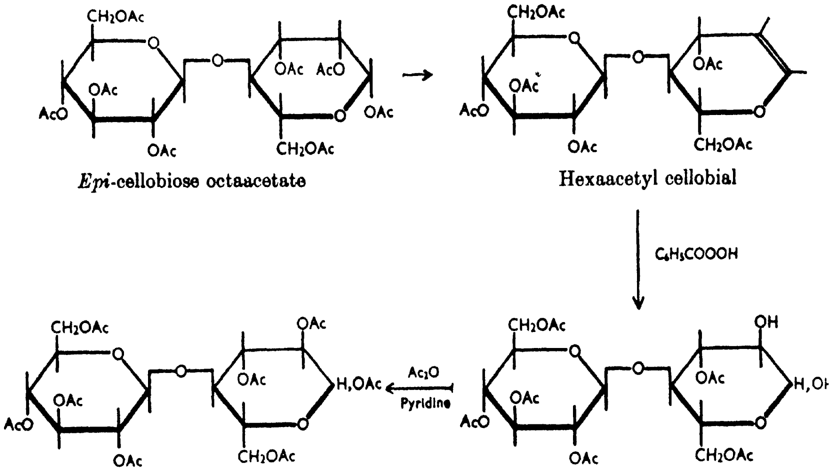
(I) (II)

---

* *Ber.*, 1933, **66**, 27.

† Gilbert, Smith and Stacey, *J.C.S.*, 1946, 622.

‡ *J. Amer. Chem. Soc.*, 1942, **64**, 1289.



Epi-cellobiose octaacetate

Hexaacetyl cellobial

Cellobiose octaacetate

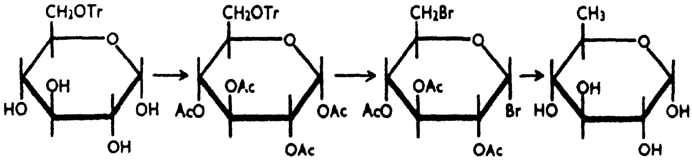
Hexaacetyl cellobiose

Lactose

Lactose has been synthesised* by a method analogous to the last method described for cellobiose except that acetobromogalactose instead of acetobromoglucose was used in the condensation.

Gentiobiose

The synthesis of the 1,6-disaccharide, gentiobiose, was accomplished at about the same time as its constitution was established. Helferich† by the use of trityl chloride (see page 52) prepared the key compound, 1,2,3,4-tetraacetyl glucose, by the acetylation of 6-trityl glucose. The structure of the product was proved to be 1,2,3,4-tetraacetyl 6-trityl glucose since on treatment with hydrogen bromide 1,6-dibromo 2,3,4-triacetyl glucose was obtained which



6-Trityl α-glucose

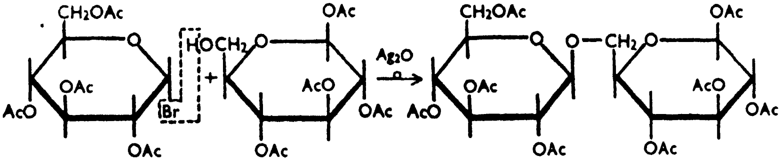
D-*Iso*rhamnose

yielded on reduction and deacetylation D-*iso*rhamnose. Both of these compounds had been prepared previously by Fischer and their isolation confirmed that the trityl group was on C_6. The trityl group was readily removed from the tetraacetyl 6-trityl glucose by treatment with acid, and condensation in chloroform solution of the 1,2,3,4-tetraacetyl glucose so obtained with acetobromoglucose in the presence of silver oxide gave octaacetyl gentiobiose. More recently the elegant sodium method* has also been used successfully for this synthesis.

---

* Haskins, Hann and Hudson, *J. Amer. Chem. Soc.*, 1942, **64**, 1852.

† Helferich and Klein, *Annalen*, 1926, **450**, 219.



Octaacetyl gentiobiose (β-form)

Melibiose

When Helferich's condensation described above was carried out with acetobromogalactose instead of acetobromoglucose, 6-[β-galactosido]-D-glucopyranose was obtained.† This substance was quite different from melibiose and supported the view that the natural disaccharide contained an α-linkage. Later† it was found that when quinoline was used instead of silver oxide for the condensation 6-[α-D-galactosido]-D-glucopyranose or melibiose could be obtained.

Vicianose, Primeverose and Rutinose

The condensation of 1,2,3,4-tetraacetyl glucose with acetobromo-L-arabinose has given vicianose,‡ with acetobromoxylose, primeverose§ and with acetobromorhamnose and 1-chloro 2,3,4-triacetyl glucose followed by suitable treatment, heptaacetyl rutinose has been synthesised.||

Iso-*Trehalose and* neo-*Trehalose*

Natural trehalose (αα′-glucopyranosido-glucopyranoside) has not been synthesised but the other two possible disaccharides of this type have. Fischer and Delbrück¶ by reacting upon acetobromoglucose with silver oxide obtained a poor yield of octaacetyl *iso*-

---

* Gilbert, Smith and Stacey, *J.C.S.*, 1946, 622.

† Helferich and Rauch, *Ber.*, 1927, **59**, 2655.

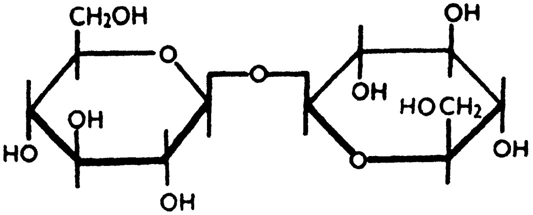
‡ Helferich and Bredereck, *Annalen*, 1928, **465**, 166.

§ Helferich and Rauch, *Annalen*, 1927, **455**, 168; McCloskey and Coleman, *J. Amer. Chem. Soc.*, 1943, **65**, 1778.

|| Zemplén and Gerecs, *Ber.*, 1934, **67**, 2049; 1935, **68**, 1318.

¶ Fischer and Delbrück, *Ber.*, 1909, **42**, 2778; McCloskey, Pyle and Coleman, *J. Amer. Chem. Soc.*, 1944, **66**, 349.

trehalose. Schlubach later obtained better results by condensing 2,3,4,6-tetraacetyl glucose with acetobromoglucose in the presence of silver carbonate and magnesium sulphate in dioxan solution. *Iso*trehalose is $\beta\beta'$-glucopyranosido-glucopyranoside.



*Iso*trehalose

The corresponding $\alpha\beta'$-compound has been prepared from Brigl's anhydride (that is 1,2-anhydro triacetyl glucose) and 2,3,4,6-tetraacetyl glucose* by direct condensation in benzene solution.

Iso*Sucrose*

No authenticated claim to have synthesised cane sugar by chemical means has yet been established. All the efforts of chemists in this direction have led to the production of the so-called *iso*sucrose. For example Irvine and his associates† condensed 2,3,4,6-tetraacetyl glucose with in one instance a tetraacetyl fructose obtained from inulin in the presence of phosphorus pentoxide, and in another experiment with a tetraacetyl fructose prepared from sucrose octaacetate.‡ Condensations were later attempted on a large scale,§ but the same disaccharide octaacetate was isolated in each case and no sucrose octaacetate could be identified, and the same result was obtained using the new technique of chromatography‖ (page 167). It is considered probable that *iso*sucrose is β-D-glucopyranosido-α-D-fructofuranoside.¶

The Enzymatic Synthesis of Disaccharides

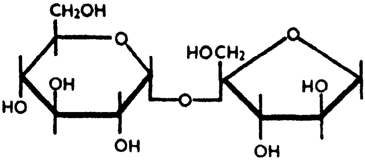
Bourquelot and Bridel showed that the hydrolysis of a glycoside, for example β-methylglucoside, with an enzyme was reversible, and that with a high concentration of the reactants, glucose and methanol, in the presence of emulsin, β-methylglucoside was produced. This method can be applied to disaccharides. Croft Hill by the action of yeast (which contains maltase) on a concentrated glucose solution obtained evidence of the formation of maltose, and, using emulsin Bourquelot prepared from glucose, gentiobiose, cellobiose and other disaccharides.* Such methods are however very inefficient. Much more illuminating are the enzymatic syntheses, using glucose-1-phosphate, of certain ketofuranosides, including sucrose, carried out in California by W. Z. Hassid, M. Doudoroff and their associates. The bacterium *Pseudomonas saccharophila* contains a phosphorylase which catalyses the reversible reaction

---

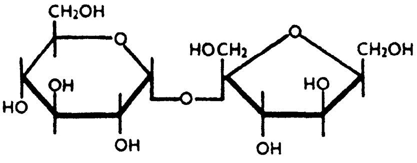
* Haworth and Hickinbottom, *J.C.S.*, 1931, 2847.
† *J. Amer. Chem. Soc.*, 1929, **51**, 1279.
‡ Irvine and Oldham, *J. Amer. Chem. Soc.*, 1929, **51**, 3609.
§ Irvine and Routledge, *J. Amer. Chem. Soc.*, 1935, **57**, 1411.
‖ Binkley and Wolfrom, *J. Amer. Chem. Soc.*, 1946, **68**, 2171.
¶ Klages and Niemann, *Annalen*, 1937, **529**, 185.

Sucrose + inorganic phosphate $\rightleftharpoons$ glucose-1-phosphate + fructose. From the dry bacteria the enzyme can be isolated and purified, and when allowed to operate in a buffered solution containing fructose and α-D-glucose-1-phosphate, crystalline sucrose can be isolated.† It may be noted that α-L-glucose 1-phosphate cannot be used as a substrate either with D- or L-fructose for disaccharide formation in this way.‡

By employing as the ketose components in the reaction mixture D-xyloketose and L-sorbose respectively, two new disaccharides, α-D-glucopyranosido-β-D-xyloketofuranoside (I)§ and α-D-glucopyranosido-α-L-sorbofuranoside (II)|| have been prepared.



(I)



(II)

Trisaccharides

Gentianose

The trisaccharide, gentianose, isolated from yellow Gentian in 1882, from which gentiobiose may be derived, is hydrolysed by enzymes, and from the products obtained its constitution can be deduced. Thus emulsin removes a molecule of glucose, and sucrose is produced, whereas the sucrose splitting enzyme invertase liberates

---

* *Compt. rend.*, 1913, **157**, 732; 1919, **168**, 1016.

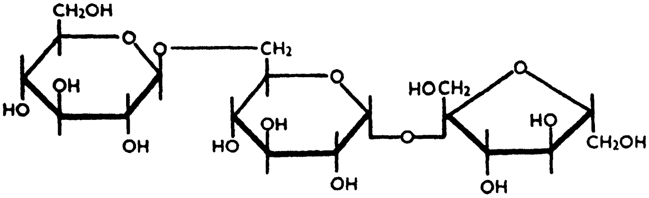
† Hassid, Doudoroff and Barker, *J. Amer. Chem. Soc.*, 1944, **66**, 1416.

‡ Potter, Sowden, Hassid and Doudoroff, *J. Amer. Chem. Soc.*, 1948, **70**, 1751.

§ Hassid, Doudoroff, Barker and Dore, *J. Amer. Chem. Soc.*, 1946, **68**, 1465.

|| Hassid, Doudoroff, Barker and Dore, *J. Amer. Chem. Soc.*, 1945, **67**, 1394.

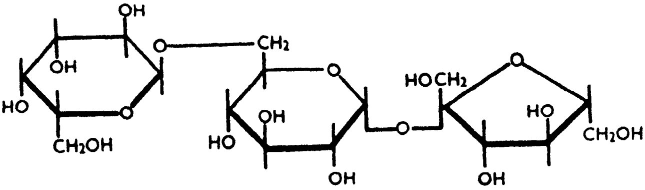
gentiobiose. The trisaccharide is non-reducing and its constitution is represented below:



Gentianose

Raffinose

From beet molasses and cotton seed hulls a non-reducing trisaccharide, raffinose, can be prepared which on hydrolysis yields galactose, fructose and glucose. By the action of invertase melibiose is produced, and emulsin yields galactose and sucrose. Nevertheless the linkage between the galactose and glucose residue is not a β-linkage, for emulsin contains in addition to a β-glucosidase an α-galactosidase. Raffinose is therefore represented as:



Raffinose

The hydrolysis of hendacamethyl raffinose gave 2,3,4,6-tetramethyl galactose, 2,3,4-trimethyl glucose and tetramethyl fructofuranose in accordance with the above formulation.*

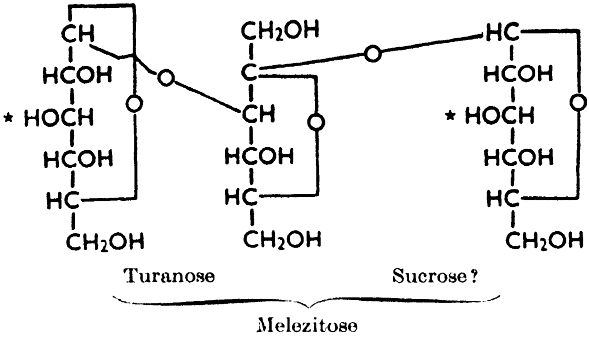
Melezitose

The trisaccharide, melezitose,† first extracted from larch manna [*la mélèze* = the larch] is found in honey dew on poplar leaves, lime trees, and as already mentioned as an exudation from the needles of the Douglas fir. Again a non-reducing trisaccharide, hydrolysis of melezitose with dilute acids liberates glucose and turanose, the former being removed by fermentation with yeast. Evidence from periodate oxidation and from enzymic hydrolysis is consistent with the composition expressed by 3-[α-D-glucopyranosido]-D- ?fructofuranosido-α-D-glucopyranoside. Although it is likely that the last two residues are united as in sucrose, no proof is, as yet available that this is so.

---

* Haworth, Hirst and Ruell, *J.C.S.*, 1923, 3125.

† See *Advances in Carbohydrate Chemistry*, 1946, **2**, 2.



By oxidation with periodic acid* it has been shown that four molecules of the reagent are used, two molecules of formic acid are liberated (from *) and no formaldehyde could be detected. This result agrees with the above representation, for formaldehyde would have been liberated if the glucose units had been furanose. The fact that only four molecules of reagent were used and only two molecules of formic acid were liberated shows also that the fructose unit could not have been in the pyranose form, and that it was not attacked by the periodic acid. This was proved further by oxidation of the tetraaldehyde produced on periodate oxidation followed by acid hydrolysis, when a laevorotatory solution was obtained which gave a good yield of glucosazone and from which the characteristic fructose *p*-nitrophenylhydrazone was isolated.

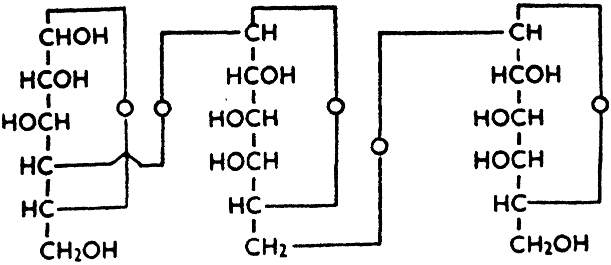
The Tetrasaccharide, Stachyose

A crystalline tetrasaccharide, stachyose, has been isolated from the manna of the ash tree and is found in the seeds of many leguminous plants. A non-reducing substance it is converted on complete hydrolysis into two molecules of D-galactose and one molecule each of glucose and fructose for every molecule of stachyose. Partial hydrolysis gives the reducing trisaccharide, manninotriose, a substance with rather a misleading name since it does not contain mannose, and which on oxidation, methylation and hydrolysis gives

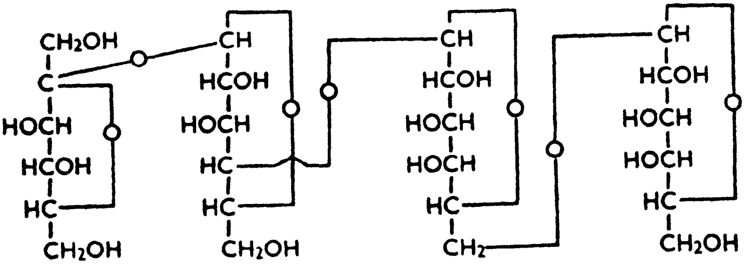
---

* Richtmyer and Hudson, *J. Org. Chem.*, 1946, **11**, 610.

2,3,5,6-tetramethyl gluconic acid, 2,3,4-trimethyl galactose and 2,3,4,6-tetramethyl galactose in agreement with the annexed formula. Stachyose is presumably derived from it by the junction of a fructofuranose unit with the terminal glucose residue as in sucrose. Fully methylated (tetradecamethyl) stachyose gave on hydrolysis 1,3,4,6-tetramethyl fructose and 2,3,4,6-tetramethyl galactose from the two end groups, 2,3,4-trimethyl galactose and a trimethyl glucose, presumably 2,3,6-trimethyl glucose since it gave tetramethyl glucopyranose on further methylation.*



Manninotriose



Stachyose

---

* Onuki, *Chem. Abstr. A.*, 1932, 1116; 1933, 378.

CHAPTER VI

POLYSACCHARIDES

The Main Structural Features of Cellulose, Starch, Glycogen, Inulin and Xylan

UNLIKE monosaccharides which are reducing, soluble in water and sweet to the taste, the polysaccharides are frequently insoluble, all are non-reducing or nearly so, and tasteless. They are made up of monosaccharide units linked together, but free hydroxyl groups are still present, as can be shown by acetylation experiments, although they are proportionately fewer in number than in the monosaccharides, namely two fewer in each monosaccharide building unit.

Most polysaccharides are found in plants, although the important reserve material glycogen is elaborated by animals, chitin is associated with the crustaceans, whilst an animal cellulose called " tunicin " is manufactured in the form of a leathery skin by ascidians and tunicates, creatures which exist in shallow sea water.

It is usual to classify polysaccharides as either skeletal, that is forming the scaffolding or structure of the plant, among which cellulose stands pre-eminent, or as reserve nutrient polysaccharides, such as starch and inulin. The expression $(C_6H_{10}O_5)_n$ is usually taken to represent a polysaccharide derived from a hexose such as glucose, for example cellulose, starch and glycogen, but such a formulation is obviously of little value in indicating either the nature, properties or constitution of the polysaccharide. It has been shown for disaccharides that a variety of different types of linkage can exist even when the hexose units are the same. Thus for the diglucoses we may have 1,4-α-links in maltose, 1,4-β-links in cellobiose, 1,6-β-links in gentiobiose whilst 1,3-β-links occur in laminaribiose. Since the molecular weight of cellulose is so high that it may contain as many as ten thousand glucose units in a single giant molecule, the difficulty of discovering how each one is linked is clearly very great. The confusion is greatly increased if, instead of a single building unit or brick such as glucose, several different units are involved in the construction, a state of affairs which exists in such compounds as gum arabic and many mucilages. Even in such complex molecules regularities of structure have been found, and the constitutions of the " classical " polysaccharides like cellulose and starch, although not worked out by any means to the last detail can at least be sketched in bold outline. It is our present purpose to indicate the main structural features of some well-known members of the polysaccharide group, leaving until later a study of the more recent developments.

As in the disaccharides, the monosaccharide units which form the building stones of the polysaccharides are united by glycosidic links which are broken on treatment with acids or with enzymes specific for the type of link to be attacked.

$$(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6$$

The polysaccharides must be classed as high polymers although the polymeric unit is not, of course, the monosaccharide unit, for example glucose, but the anhydro-hexose residue $C_6H_{10}O_5$. Such a conception is however purely artificial since an anhydroglucose unit which could polymerise to give a polysaccharide has never been isolated, and it is better to regard the polysaccharide group as formed by condensation polymerisation of the monosaccharide units.

Until recently the only sure method of attacking the problem of polysaccharide structure was that exploited with such success by Haworth and his School, of methylation followed by hydrolysis and the separation and identification of the various methylated fragments. This method is still the most valuable one but another weapon of attack, periodate oxidation, is finding many applications.

Cellulose

Cellulose* is the chief structural polysaccharide of plants, and is the raw material of many industries, with a tremendous output in the form of cotton goods, paper, jute, cellulose, rayon, explosives and plastics. It varies in purity with its origin, although cotton cellulose requires but little treatment before it can be regarded as nearly pure cellulose. Whereas cellulose is digested by ruminants and horses, it is not digested by human beings. An enzyme capable of hydrolysing regenerated cellulose (that is cellulose which has been dissolved and reprecipitated, such as viscose rayon) into glucose has been isolated by Karrer from the snail *Helix pomatia*, but this enzyme is not capable of hydrolysing cotton to an extent of more than about 30%.

---

* See E. Heuser, *The Chemistry of Cellulose* (John Wiley & Sons, New York, 1944); Marsh and Wood, *Introduction to Cellulose Chemistry* (Chapman & Hall, London, 1945); E. Ott, *Cellulose and Cellulose Derivatives* (Interscience Publishers Inc., New York, 1943).

As far as we know at present, the constitution of cellulose is the same, whatever its source. Thus the animal cellulose, tunicin, is chemically indistinguishable from plant celluloses.* A bacterial cellulose produced by the action of *Acetobacter xylinum* in nutrient solutions containing glycerol, glucose or fructose, has been exhaustively investigated and found to be both chemically identical with cotton cellulose† and to give a closely similar X-ray diffraction pattern. At the same time, since we are dealing with a giant molecule, it must be borne in mind that the present chemical methods of attack are not sufficiently delicate to prove with certainty that the "fine structure" of all celluloses is the same.

The only sugar which has been isolated on the hydrolysis of cellulose is glucose. It must be admitted that the yield of crystalline glucose has never reached the theoretical value, but Monier-Williams‡ by hydrolysis with sulphuric acid isolated nearly 91% of crystalline glucose and yields of almost 96% have been obtained from wood pulp.§ It is not to be expected that theoretical yields of glucose would be obtained from the hydrolysis of such a resistant molecule as cellulose, since on treatment with hot acids glucose gives rise to breakdown products such as levulinic and formic acids. Polarimetric observations on cellulose hydrolysed at room temperature by concentrated hydrochloric acid have given confirmation of the view that glucose is the only sugar produced on hydrolysis. By the action of methanolic hydrogen chloride on cellulose acetate a yield of methylglucosides amounting to 95% of the theoretical has been obtained.‖

The elementary analysis of cellulose leads to the empirical formula $C_6H_{10}O_5$, and the fact that, based on this unit, a triacetate and a trimethyl ether can be prepared shows that three free hydroxyl groups per glucose unit are present. 2,3,6-Trimethyl glucose was isolated from a partly methylated cellulose by Denham and Woodhouse¶ and a fully methylated cellulose was hydrolysed by Irvine and Hirst[*] to give 2,3,6-trimethyl glucose in a yield of 86%. Later workers showed that a small quantity of tetramethyl glucopyranose was produced as well, and the significance of this fact will be discussed later.

---

* Zechmeister and Tóth, *Z. physiol. Chem.*, 1933, **315**, 267.

† See Evans and Hibbert, *Advances in Carbohydrate Chemistry*, 1946, **2**, 206 Academic Press, New York.

‡ *J.C.S.*, 1921, 803.

§ Heuser and Aiyar, *Z. angew. Chem.*, 1924, **37**, 27.

‖ Irvine and Soutar, *J.C.S.*, 1920, 1489; Irvine and Hirst, *J.C.S.*, 1922, 1585.

¶ *J.C.S.*, 1914, 2357.

Only two possibilities exist, therefore, for the manner in which the glucose units are linked, namely through C_1 and C_4 (in which case the rings must be pyranose) or C_1 and C_5 (in which event glucofuranose units would be involved). This latter possibility on the face of it is very doubtful if we bear in mind the extreme ease with which glucofuranosides are hydrolysed by dilute acids, inasmuch as cellulose is relatively resistant to hydrolysis.

The isolation of cellobiose octaacetate by acetolysis is of great significance. Although the maximum yield is only slightly more than 50% of the theoretical[†] the reaction is such that the maximum yield based on calculations of probability for a chain of one hundred glucose units has been computed to be 67%[‡] but since cellobiose itself is hydrolysed under the conditions of the experiment such a high yield is not to be expected. The structure of cellobiose was worked out as described in the previous chapter as 4-[β-D-glucopyranosido]-D-glucopyranose, from which it would seem to follow that cellulose contains similar linkages. The only objection to such a hypothesis would be if cellobiose were what is known as a reversion product, if for example another unstable disaccharide were produced initially during the acetolysis, which then changed its structure to cellobiose, in much the same way that the fructofuranose unit in sucrose is isolated as fructopyranose. There were indeed proponents of this idea but it is unnecessary to discuss the arguments since they have proved erroneous. If it is admitted that cellobiose represents a true degradation product of cellulose, it follows that the glucose residues are united by 1,4-β-links in the polysaccharide.

In addition to cellobiose, authenticated tri- and tetra-saccharides have been isolated by the partial hydrolysis of cellulose. Using concentrated hydrochloric acid Zechmeister and Tóth[§] isolated a cellotriose, a cellotetraose and other products which, from the low values of their optical rotations must contain β-linkages.

Evidence of great value was provided by Haworth, Hirst and

---

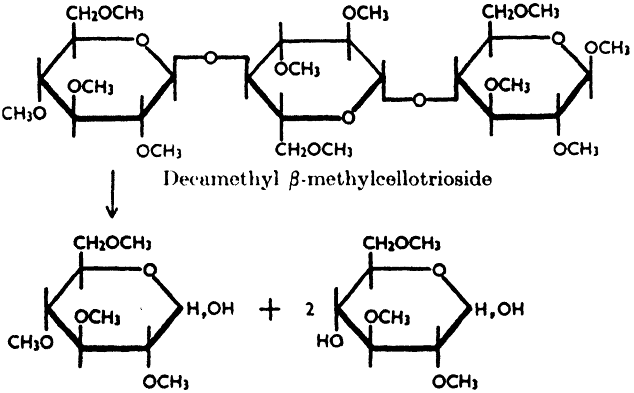
[*] *J.C.S.*, 1923, 518.

[†] Friese and Hess, *Annalen*, 1927, **456**, 38.

[‡] Freudenberg, *Ber.*, 1921, **54**, 767; Karrer and Widmer, *Helv. Chim. Acta*, 1921, **4**, 174.

[§] *Ber.*, 1931, **64**, 854.

Thomas* who subjected fully methylated cellulose to acetolysis at the low temperature of 15°, for a period of two hours, thereby diminishing to a minimum any possibility of rearrangement. This was followed by methylation to substitute any acetylated hydroxyl groups exposed during the hydrolytic cleavage, and by distillation under diminished pressure, after removal of tetramethyl methylglucosides, crystalline heptamethyl β-methylcellobioside was isolated together with a crystalline product which proved to be a decamethyl β-methylcellotrioside. This latter substance on hydrolysis gave tetramethyl glucopyranose (1 part) and 2,3,6-trimethyl glucose (2 parts). Without question, therefore, methylated cellulose contains at least three contiguous glucopyranose units. By condensing the chlorhydrin of heptamethyl cellobiose with 2,3,6-trimethyl β-methyl glucoside Freudenberg and Nagai† synthesised the methylated cellotriose concerned.



Decamethyl β-methylcellotrioside

Corroborative evidence that cellulose consists of a chain of glucopyranose units linked through C_1 and C_4 is secured from many studies on the kinetics of the hydrolysis of cellulose by acids. Comparison of the rates of hydrolysis of cellulose, cellobiose, starch and maltose in a zinc chloride-hydrochloric acid solution shows that the rates of hydrolysis for the first two substances were comparable and much slower than for the last two.‡ Maltose is, in fact, hydrolysed more than twice as rapidly as cellobiose under the same conditions, so that α-linkages, if present in cellulose, should be detectable with ease.

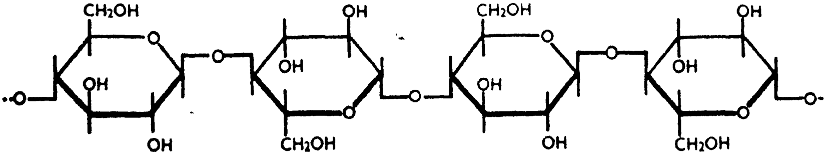
---

* *J.C.S.*, 1931, 824.

† *Annalen*, 1932, **494**, 63.

‡ Hibbert and Percival, *J. Amer. Chem. Soc.*, 1930, **52**, 3995.

Mathematical analyses of the rate of hydrolysis have been carried out by W. Kuhn* and show that the results are best explained by the hydrolytic cleavage of one type of linkage only, the β-link. Most measurements of the rate of hydrolysis of cellulose have been carried out by polarimetric observation or by determinations of reducing power, but an ingenious method due to Wolfrom and Georges† may be mentioned also. By combining the products of hydrolysis with ethyl mercaptan any free reducing groups react to form the open chain thioacetal (see page 47) $-\mathrm{CH(OH)}\,.\,\mathrm{CH}{<}^{\mathrm{SEt}}_{\mathrm{SEt}}$ and by an analysis for sulphur of the products at each stage, it is possible to follow the gradual diminution in length of a cellulose molecule.‡ Thus on hydrolysis in fuming hydrochloric acid at 16°, cotton linters after 30 minutes had an average of 89 glucose units in a chain compared with 23 after 150 minutes. It must be emphasised, however, that the values represent an average. All the hydrolytic methods, nevertheless, are in harmony with the idea of a chain structure, built up on the basis of a cellobiose unit, which under the action of acids is broken down at the glycosidic links in a perfectly regular manner.



Chain of β-D-Glucopyranose Units in Cellulose

X-ray analysis has provided striking evidence of the essential correctness of the above ideas. The fact that an X-ray pattern is obtained at all is evidence of some kind of orientation of structure. Crystals, as is well known, give X-ray diffraction patterns, but the mechanical properties of cellulose are by no means those of a crystal. The only other possible explanation is a regular arrangement or pattern of some kind and parallel bundles of long chains would explain, qualitatively at any rate, the observed results. The results can also be interpreted quantitatively in a remarkable way. The early X-ray measurements of Polanyi§ probably attracted little attention, although his measurements were used later by other workers. It was the work of Sponsler and Dore[*] which really focussed attention on this aspect of the subject. Studying the X-ray diffraction pattern of ramie cellulose these workers calculated the length of the cell along the fibre axis to be 10·25Å. This was found to agree exactly with the length of two glucose units joined by an oxygen atom. The length of one glucose unit calculated from accepted values of bond lengths is found to be 5·13Å *provided* the six atom or pyranose ring of Haworth is adopted but not if the furanose structure is concerned. A difficulty arose in the interpretation of Sponsler and Dore, however, because they considered their results best expressed in terms of a formula containing glucopyranose units linked alternately through 1,1 and 4,4 positions. Haworth dissented from this view, since ether and glucosidic links appeared alternately, and the isolation of cellobiose was ignored. Others, notably Meyer and Mark,[†] seized upon Haworth's suggestion that 1,4-links were present, and, by the use of the X-ray measurements of Polanyi[‡] were able to fit five cellobiose residues into a unit cell. Later measurements[§] have only modified these conclusions slightly and the present view is that the unit cell has the dimension *a* (horizontal)

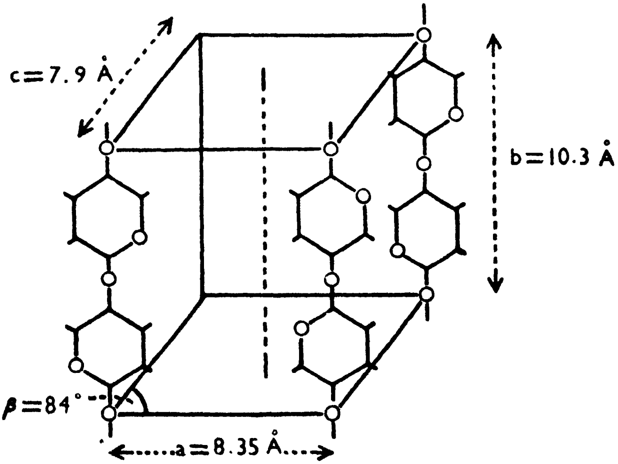
---

* See Freudenberg, *J. Soc. Chem. Ind.*, 1931, **50**, 287 T.

† *J. Amer. Chem. Soc.*, 1937, **59**, 282.

‡ Wolfrom, Georges and Sowden, *J. Amer. Chem. Soc.*, 1938, **60**, 1026.

§ *Z. Physik.*, 1921, **7**, 149.



Unit Cell of Cellulose showing three of the five cellobiose residues

---

[*] *Colloid Symposium Monograph*, 1926, **4**, 174. New York.

[†] *Ber.*, 1928, **61**, 593.

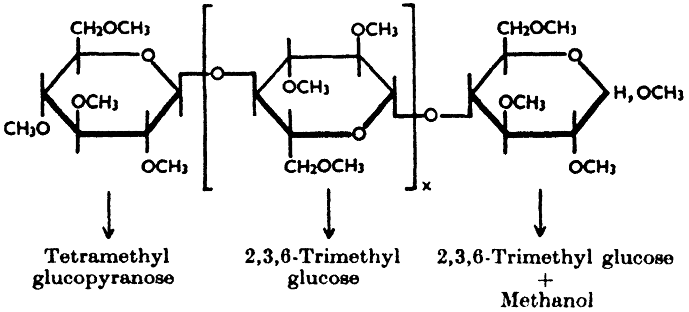
[‡] *Z Physik*, 1921, **7**, 149.

[§] Meyer and Misch, *Helv. Chim. Acta*, 1937, **20**, 232.

8·35Å, *b* (vertical) parallel to fibre axis, 10·3Å, *c* (at an angle β with *a*) 7·9Å, $\beta = 84°$. The chains of glucose residues, in groups of five in a crystallographic unit, are, according to Meyer and Misch, supposed to point alternately in opposite directions.

Once the idea of cellulose as a chain molecule was established it was natural to pursue the enquiry to see how long the chains were and what groups were to be found at the ends. Cellulose is only very slightly reducing, and on the hydrolysis of trimethyl cellulose only 2,3,6-trimethyl glucose had been isolated. These facts explain, in part at any rate, the disposition of many workers to represent cellulose by cyclic structures. Obviously a chain of glucose units, provided it is not in the form of a loop, must have an end group which, if the specimen is methylated, will give tetramethyl glucopyranose on hydrolysis, and at the other end, one would expect to find a glycosidic methoxyl attached to the reducing group. By the hydrolysis of a specimen of methylated cellulose with concentrated hydrochloric acid, and careful fractionation of the products by distillation in a high vacuum, using an efficient fractionating column designed by Widmer, Haworth and Machemer* isolated 0·6% of tetramethyl glucopyranose from 200 g. of methylated cellulose. This was the first demonstration that cellulose had a terminated chain, or to be more precise that this was the state of affairs in the specimen of methylated cellulose which was examined. From the proportion of tetramethyl glucopyranose isolated in this way, it was calculated that the *average* chain length of the methylated cellulose hydrolysed lay between 100 and 200 glucopyranose units. Since the specimen had been prepared from a cellulose

The Hydrolysis of Methylated Cellulose



---

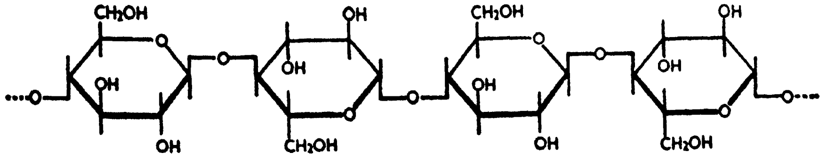
* *J.C.S.*, 1932, 2270.

acetate, made from cotton linters by acetylation with acetic anhydride in the presence of an acid catalyst, it is clear that the result would not be expected to be the same as for native cellulose, the probability being that some degradation must have occurred. Haworth recognised this fact and also that the result was really only an average value of the chains of varying length present in his preparation. Nevertheless this result represents a great advance both in practice and in theory, and demonstrates that cellulose could not have a chain length smaller than about two hundred glucose units.

Another important contribution followed* in which cellulose was degraded by acetolysis and the various cellodextrin acetates were fractionated. By treatment with alkaline sodium hypoiodite it was possible to determine the average chain length of these smaller molecules (which varied between eleven and twenty-one glucose units), since the *reducing groups* were exposed and the molecular length could be estimated by a reaction, which is a standard method† for estimating aldoses.

$$\mathrm{RCHO + NaIO + NaOH = RCOONa + NaI + H_2O}$$

The same samples on methylation and hydrolysis gave weights of tetramethyl glucopyranose from the *non-reducing* end of the chains which should also have corresponded to the chain length. In most instances the results of the two sets of determinations were in good agreement, giving support both to the chain theory and to the method of gravimetric assay of chain length by the weight of tetramethyl glucose obtained. The subject of the molecular weight of cellulose and other points such as the interaction of the individual chains of glucose units will be considered later, but for the present it will be sufficient to depict cellulose by very long chains of β-glucopyranose units linked through the first and fourth positions.



Chain of β-D-Glucopyranose Units in Cellulose

---

* Haworth and Machemer, *J.C.S.*, 1932, 2372.

† Bergmann and Machemer, *Ber.*, 1930, **63**, 316, 2304.

Lichenin

A water soluble polysaccharide resembling cellulose in its chemical constitution, called lichenin is found in Iceland Moss (*Cetraria Islandica*). Composed entirely of D-glucose units, cellobiose octaacetate has been obtained from it on acetolysis,[*] and 2,3,6-trimethyl glucose was obtained by the hydrolysis of methylated lichenin.[†]

Investigations of molecular size, however, have revealed that the chain length, estimated by the chemical end group assay is much smaller than in cellulose, a value of about 80 glucopyranose units having been deduced. Molecular weight estimations by the osmotic pressure method have given values of the same order, ranging between 80 and 160 units,[‡] so the evidence indicates that lichenin has a smaller molecule than cellulose: this is not surprising since lichenin is soluble in water.

It has been shown recently that about thirty per cent of the linkages in lichenin are 1,3 and the remainder 1,4. This conclusion is based on an examination of the trimethyl glucoses produced on hydrolysis of fully methylated lichenin using the periodic acid technique, 2,4,6-trimethyl glucose being unaffected by the reagent.[§] Confirmatory evidence has been supplied by the reduction of the trimethyl glucoses to the corresponding glucitols and chromatographic separation of the resulting coloured compounds, 1,4,5-triazoyl 2,3,6-trimethyl glucitol and 1,3,5-triazoyl 2,4,6-trimethyl glucitol.[‖]

Starch

The substance usually called starch is, almost invariably, a mixture of polysaccharides, forming the reserve food material of most plants, and one of the principal articles of diet since, unlike cellulose, it is hydrolysed, and therefore digested, relatively easily. Starch usually occurs as granules which differ in size and shape from plant to plant, and are, as a rule, easily identified by their appearance under the microscope, where the inner nucleus or hilum is seen surrounded by striations representing concentric layers.

Generally speaking the chemical properties of starch from various sources, and its derivatives, appear to be the same, although variable but small quantities of phosphorus are contained in most natural starches, a fact of significance, as will appear later, in connexion with the building up of starches by enzymes.

---

[*] Karrer and Joos, *Biochem. Z.*, 1923, **136**, 537.

[†] Karrer and Nishida, *Helv. Chim. Acta*, 1924, **7**, 363.

[‡] Carter and Record, *J.C.S.*, 1939, 624.

[§] Meyer and Gürtler, *Helv. Chim. Acta*, 1947, **30**, 751.

[‖] Boissonnas, *ibid.*, 1703. Azoyl = *p*-phenylazobenzoyl.

As mentioned above starch is not a single polysaccharide (with the possible exception of waxy maize starch extracted from " waxy " maize and similar varieties) but a mixture of two components called amylose and amylopectin. A somewhat incomplete separation may be carried out by swelling the starch granules with warm water, at about 70° so that the granule does not burst, the amylose dissolving and diffusing out of the interior of the granules. After separation, however, the amylose undergoes what is known as retrogradation, becoming less soluble than the amylopectin and it is precipitated from solution on standing for long periods. This effect is observed most strongly when the separation of amylose from amylopectin is most complete and is due to the orientation and aggregation of long chains. The average proportion of amylose is about 20% of the whole starch, as in the potato, but certain starches such as waxy maize starch contain practically no amylose. Recently much more satisfactory methods have been devised for the separation of amylose using chloral hydrate, thymol and *n*-butanol. By the application of various techniques with the latter material Schoch[*] has isolated a crystalline amylose composed of six-leaved rosettes of crystals and Kerr and Severson[†] have obtained single crystals of Indian corn amylose.

Of the general properties of amylose it may be said at this stage that it is characterised by giving a deep blue colour with iodine, much deeper than that given by whole starch, and that amylose is hydrolysed completely to maltose in contact with β-amylase.

Amylopectin which remains behind after the amylose has diffused out, makes up about 80% of the starch granule. Unlike amylose this constituent gives viscous aqueous solutions and is responsible for the formation of starch pastes. The amylopectin constituent gives a reddish colour with iodine and is only converted into maltose by β-amylase to the extent of about 50%, the residue being a dextrin.

For the present it is proposed to ignore the differences between the two starch constituents, since certain fundamental features are common to both polysaccharides, and no difficulty arises until we come to considerations of detail. Much of the earlier work was necessarily carried out using whole starch, which, as we have explained, is mainly amylopectin, since it is only comparatively recently that pure amylose has been isolated and investigated in detail, and the expression "starch" will be used generally, except when specific separations have been achieved.

---

[*] *J. Amer. Chem. Soc.*, 1942, **64**, 2957.

[†] *J. Amer. Chem. Soc.*, 1943, **65**, 193.

On hydrolysis with acids starch gives a quantitative yield of glucose, and no other sugar can be detected among the products of hydrolysis. Analysis gives the empirical formula, $C_6H_{10}O_5$ and, like cellulose, starch can be converted into trisubstituted derivatives, such as the triacetate. Fully methylated (trimethyl) starch on hydrolysis gives a good yield of the crystalline 2,3,6-trimethyl glucose.* As already mentioned enzymic hydrolysis with β-amylase gives maltose, the yield from the whole starch being approximately 80%. Such a result is indicative that starch might be constructed on the maltose pattern, that is with α-D-glucopyranose units linked through positions 1 and 4, but reactions involving the use of enzymes are open to suspicion since, as mentioned in Chapter V, the synthesis of disaccharides can be carried out using enzymes. While admitting this, it should be stated that such suspicions are hardly justifiable in the present instance, both on account of the high yield of maltose obtained from starch and because the optimum conditions for hydrolysis and for synthesis in such a reversible reaction would be expected to differ very considerably. Karrer† by treating starch with acetyl bromide obtained heptaacetyl maltosido-1-bromide which was converted into heptaacetyl maltose, thus providing chemical evidence that maltose could be obtained from starch.

Proof was obtained‡ that two contiguous glucopyranose units joined as in maltose existed in the starch molecule by the acetolysis of methylated starch with acetyl bromide in chloroform solution at room temperature. After the removal of combined bromine by silver carbonate the mixture of products was oxidised, methylated and separated by distillation in a high vacuum. This process gave methyl octamethyl maltobionate (I) in 22% yield, which on hydrolysis yielded tetramethyl glucopyranose (II) and tetramethyl γ-gluconolactone (III) as shown on page 115.

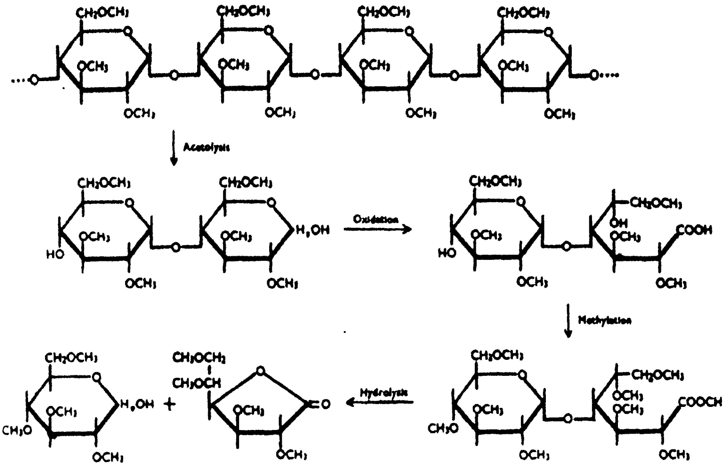
It will be recalled that the same products were obtained from maltose, so that the experiment proves that at least 21% of the methyl-

---

* Haworth, Hirst and Webb, *J.C.S.*, 1928, 2681.

† Karrer and Nägeli, *Helv. Chim. Acta*, 1921, 4, 263.

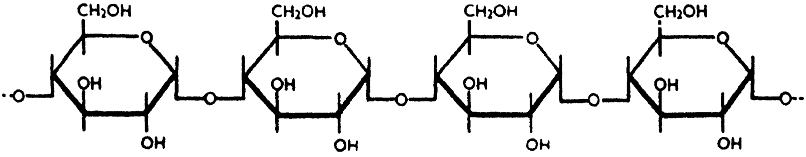
‡ Haworth and Percival, *J.C.S.*, 1931, 1342.



(II) (III) (I)

ated starch was made up of maltose residues. As in the preparation from cellulose of cellobiose, however, one could not expect all the methylated starch to be converted into a maltose derivative; the process is one of continuous hydrolysis, some maltose residues being hydrolysed further to glucose while units of longer chain length are also present. Owing to the low temperature of the experiment the possibility that the maltose derivatives isolated were reversion products can be ignored.

The idea developed gradually therefore, that starch is composed of a chain of glucose units linked, as in maltose by α-glycosidic bonds. Such a view is supported by the high values of the optical



Chains of α-D-Glucopyranose Units as in Starch

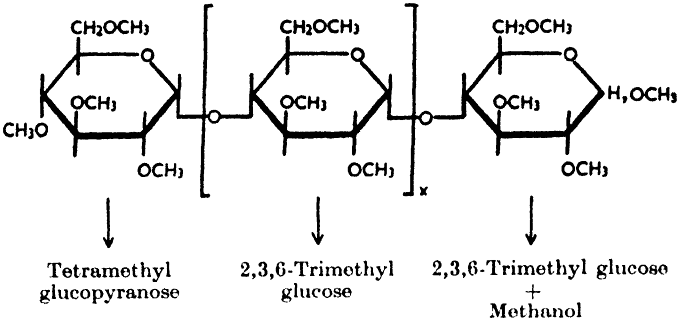
rotation of starch derivatives—methylated starch has $[\alpha]_D = +215°$. Experiments on the comparative hydrolysis of starch and maltose* also lend support to this view.

Having established the general picture with reasonable certainty, the next step was to decide if starch had an open chain or a loop structure, and between 1932 and 1939 many determinations of the proportion of tetramethyl glucopyranose obtained by the hydrolysis of various methylated starches were made. The quantities obtained were surprisingly large, amounting to about 4·5%, or seven times as much as Haworth and Machemer had obtained from methylated cellulose; the value of x thus lies between 20 and 30. A number of results are collected below to indicate the variety of starches examined.

---

* Hibbert and Percival, *J. Amer. Chem. Soc.*, 1930, **52**, 3995.

The Hydrolysis of Methylated Starch



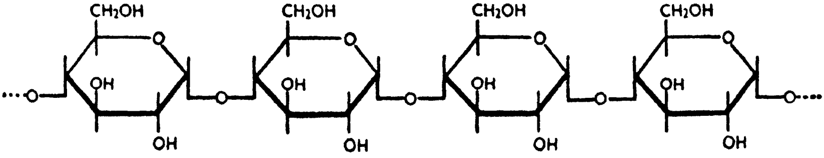
Source	*Chain length*	*Authors*
Potato	24	Hirst, Plant and Wilkinson, 1932
Potato amylopectin	24	Hirst, Plant and Wilkinson, 1940
Potato	25	Baird, Haworth and Hirst, 1935
Potato amylopectin	25	McCready and Hassid, 1943
Potato amylopectin	25	K. H. Meyer, Wertheim and Bernfeld, 1940
Waxy Maize	26-30	Haworth, Hirst and Woolgar ,1935
Maize	25	K. H. Meyer *et al*, 1941.
Maize (Golden Bantam)	25	Hassid and McCready, 1941
Canna	27	Hassid and Dore, 1937
Wheat	24	Hirst and Young, 1939
Horse Chestnut	28	Hirst and Young, 1939
Rice	30	Hirst and Young, 1939
Banana	24	Hawkins, Jones and Young, 1940

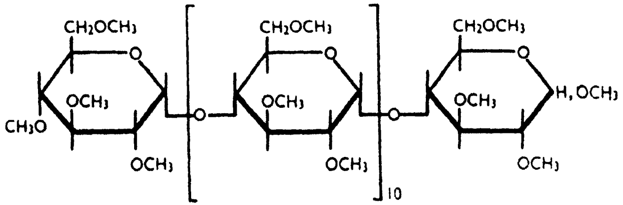
Superficially therefore, it would appear that starch, or at any rate amylopectin which comprises the bulk of the starch grain, is to be represented as a molecule containing about twenty-five glucose units in a chain, with a molecular weight of about 4000. Such an idea can be dismissed almost at once since it has been known for a long time that the molecular weight of starch is far higher than 4000. A second difficulty lies in the fact that it should be possible to measure the reducing power of such a starch either with Fehling's solution or alkaline hypoiodite and a value of approximately one twenty-fifth of the value for glucose should be obtained for the small molecule envisaged above. In practice, however, the reducing power of starch does not approach this figure. A discussion of the subject of the detailed structure and molecular weight of starch will be found in the following chapter.

Glycogen

The reserve carbohydrate of animals is glycogen; it is stored in the liver and undergoes conversion to lactic acid in the muscles during exercise. In passing it is interesting to note that a substance resembling glycogen has been isolated from the seed of sweet corn.* Unlike starch, glycogen is soluble in water, and its solution gives a brownish red colour with iodine. Elementary analysis agrees with the formula $C_6H_{10}O_5$ and hydrolysis with acids gives glucose exclusively. β-Amylase causes glycogen to be converted into maltose in a yield of about 50% so that in this respect it resembles amylopectin (page 113).

Methylated glycogen like methylated starch has a high positive specific rotation and the degradation of methylated glycogen† with acetyl bromide followed exactly the same course as for methylated starch, giving an almost identical yield of methyl octamethyl maltobionate. Glycogen is therefore to be regarded as built up in the same fundamental pattern as starch.





Methylated glycogen

An important difference emerged, however, when methylated rabbit liver glycogen was hydrolysed, in the end group assay‡ for the yield of tetramethyl glucose was double that obtained from methylated starch, namely 9%. This corresponds to a chain length of twelve units. Later it was found that in some specimens of glycogen the yield of tetramethyl glucopyranose amounted to 6·7%, corresponding to a chain length of eighteen glucose units. The following table gives a list of some of the results obtained for various specimens:

---

* Morris and Morris, *J. Biol. Chem.*, 1939, **130**, 535; Hassid and McCready, *J. Amer. Chem. Soc.*, 1941, **63**, 1632.

† Haworth and Percival, *J.C.S.*, 1931, 1342.

‡ Haworth and Percival, *J.C.S.*, 1932, 2277.

Source of Glycogen	*Chain length*	*Authors*
Rabbit (liver)	12	Haworth and Percival, 1932
Rabbit (liver)	12	D. J. Bell, 1935
Fish (*Gadidae;* livers)	12	D. J. Bell, 1935
Rabbit (liver, galactose fed)	18	D. J. Bell, 1936
Rabbit (liver, sucrose fed)	18	Baldwin, Bacon and D. J. Bell, 1944
Mussel (*mytilus edulis*) (whole tissues)	18	D. J. Bell, 1936
Rabbit (liver)	18	Haworth, Hirst and Isherwood, 1937
Horse (muscle)	12	D. J. Bell, 1937
Dog (liver)	12	Hassid and Chaikoff, 1938
Dogfish (liver)	12	Haworth, Hirst and Smith, 1939
Haddock (liver)	12	Haworth, Hirst and Smith, 1939
Hake (liver)	12	Haworth, Hirst and Smith, 1939
Dogfish (muscle)	12	Haworth, Hirst and Smith, 1939
Snail (*Helix*; whole body)	12	Baldwin and D. J. Bell, 1940
Maize (golden bantam seed)	12	Hassid and McCready, 1941
Yeast	12	Jeanloz, 1944
Mussel (muscle)	12	Meyer, Prins and Jeanloz, 1941-43
Ascaris lumbaricoides	12	Halsall, Hirst and Jones, 1945
Human (muscle)	11	Halsall, Hirst and Jones, 1945
Rabbit (liver)	18	Halsall, Hirst and Jones, 1945
Rabbit (fasted; muscle)	13	Halsall, Hirst and Jones, 1945

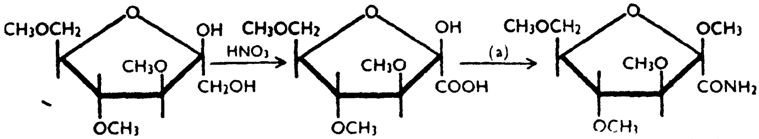
From the above list the arrangement of twelve glucopyranose units in a chain is seen to be the more usual.

Since determinations of molecular weight by physical methods reveal that the value for glycogen is certainly more than a million, it is obvious that the relatively small unit calculated from the end group assay is multiplied many times in the polysaccharide structure; the elaboration of this point will be deferred until a later chapter.

Inulin

The roots of the *Compositae*, the largest group of flowering plants, contain a reserve carbohydrate known as inulin, good sources being artichokes, chicory, dahlia tubers, and dandelion roots.

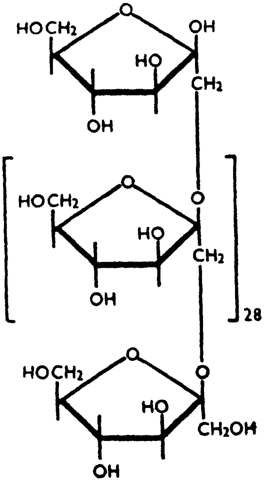
A high yield of D-fructose is obtained by the hydrolysis of inulin, although many workers have isolated, in addition a small quantity of D-glucose.* By the hydrolysis of methylated inulin, the analytical composition of which agrees with the formula for a trimethyl compound $[C_6H_7O_2(OCH_3)_3]_x$, Haworth and Learner† isolated 3,4,6-trimethyl fructose. This structure was assigned for the following reasons. The absence of a methoxyl group on C_1 was shown by the formation of a crystalline trimethyl fructosazone. Cautious oxidation with nitric acid followed by simultaneous ester and methylfructoside formation and conversion to the amide gave the same crystalline 2,3,4,6-tetramethyl 2-keto-gluconamide as that previously obtained from tetramethyl fructofuranose (see page 93). Inulin, inulin acetate, and methylated inulin, all give laevorotatory solutions, so that it appears highly probable that the polyfructosan is made up principally of fructofuranose units linked β-glycosidically from the hydroxyl group on C_2 to the primary alcoholic group on C_1. It will be observed that in inulin as in sucrose, the fructose residues are furanoid in character; in keeping with this is the property possessed by both compounds, of extreme ease of hydrolysis with traces of acids. Comparative hydrolyses of inulin and sucrose showed that inulin was hydrolysed at 65° by oxalic acid at a rate of the same order as that of sucrose.‡ Attempts to purify inulin and obtain a



3,4,6-Trimethyl fructofuranose

2,3,4,6-Tetramethyl 2-ketogluconamide

(a) = $CH_3OH - HCl$ followed by NH_3



Inulin

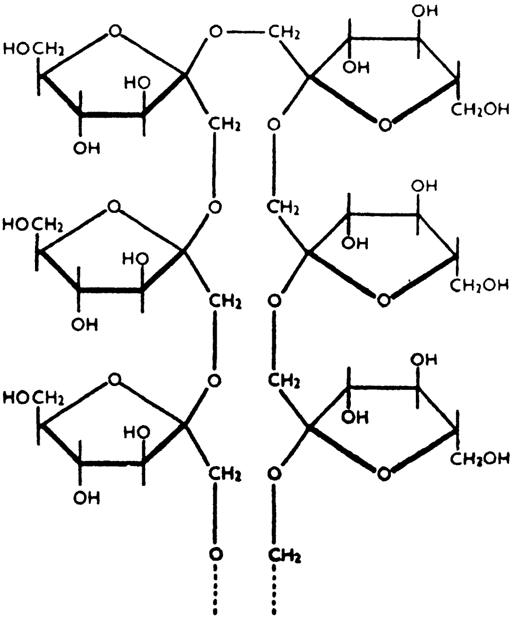
---

* See E. J. McDonald, *Advances in Carbohydrate Chemistry*, 1946, **2**, 256, Academic Press, New York.

† *J.C.S.*, 1928, 619.

‡ Hibbert and Percival, *J. Amer. Chem. Soc.*, 1930, **52**, 3995.

product not reducing to Fehling's solution were unsuccessful.* By the ebullioscopic method in water, Drew and Haworth determined the minimum molecular weight to be not less than 3200 or 3600, which corresponds to a molecule containing 20 or 24 fructofuranose units. An " end group assay " of the chain length gave 3·7 % of tetramethyl fructofuranose, corresponding to a minimum chain length of thirty fructose units.† By measurements of the osmotic pressure of acetylated and methylated inulin, values of 8880 and 6210 corresponding with the above chain length, have been obtained,‡ so that the values obtained by this fundamental physical method are in good agreement with the results of the chemical and group assay. Owing to the extreme ease with which inulin is hydrolysed even by boiling water, it is impossible to say from the evidence available if the polysaccharide exists in the plant in a cyclic form as suggested by Schlubach, although such a structure can be easily imagined from the following formulation:



Inulin

---

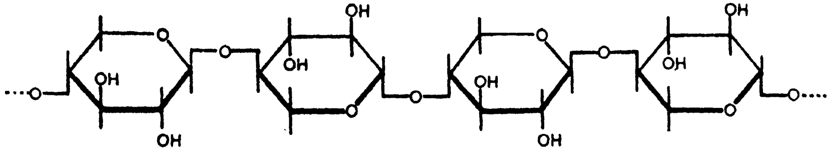
* Drew and Haworth, *J.C.S.*, 1928, 2090.

† Haworth, Hirst and Percival, *J.C.S.*, 1932, 2384.

‡ Carter and Record, *J.C.S.*, 1939, 624.

Xylan, A Pentosan

As a representative of the important group of pentosans, we will consider xylan, which is found associated with cellulose in wood (hence the name " Holzgummi "), straw, and particularly in esparto grass, an important raw material for fine paper manufacture. Xylan may be extracted from esparto, in which it occurs to the extent of 25-30%, by aqueous sodium hydroxide solution, the polysaccharide being precipitated in alcohol acidified with acetic acid. Hydrolysis with nitric acid gives a high yield of D-xylose (93%) and the polysaccharide may be represented crudely by $(C_5H_8O_4)_x$. The hydrolysis of methylated xylan (dimethyl xylan) gave a dimethyl xylose* which was proved to be the 2,3-dimethyl derivative as follows: Methylation and hydrolysis gave 2,3,4-trimethyl xylose so that there could have been no methyl group on C_5. Oxidation gave a dimethyl xylonic acid from which a typically slowly hydrolysed γ-lactone was obtained, and which on methylation gave 2,3,5-trimethyl xylonolactone. Clearly therefore C_4 was unoccupied by methoxyl in the dimethyl xylose concerned, which must have been 2,3-dimethyl xylose. Evidence that C_2 was in fact occupied was also available from the fact that no osazone could be prepared from the dimethyl sugar. The strong negative rotation of xylan in sodium hydroxide solution, and of its derivatives was evidence that β-links were present, and, on the general grounds of the stability of xylan, a formula made up of xylopyranose units linked by β-links through C_1 and C_4 was suggested.*

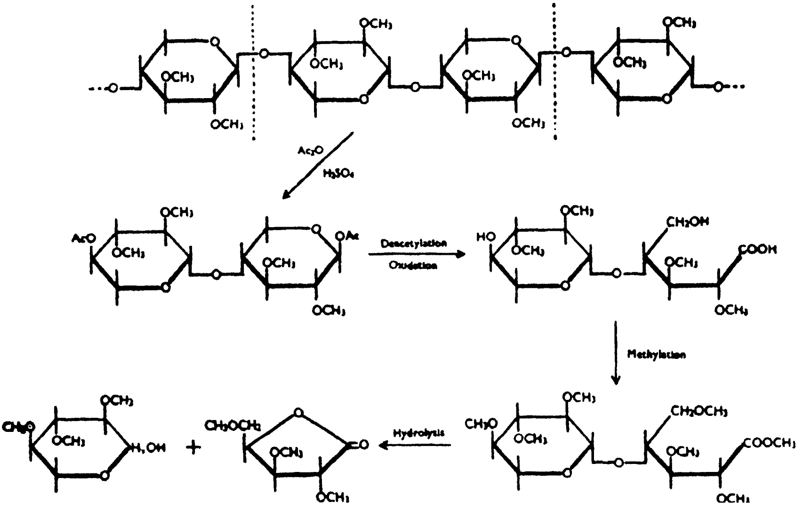


Chain of β-D-Xylopyranose Units as in Xylan

Proof that two adjoining xylose units were pyranose was obtained by acetolysis experiments on dimethyl xylan, experiments similar in character to those described previously for starch and glycogen. Degradation with acetic anhydride containing sulphuric acid during the short interval of ten minutes at room temperature, deacetylation, oxidation, methylation and separation of the disaccharide portion (21%) by distillation in a high vacuum gave a hexamethyl methylxylobionic ester. Hydrolysis yielded trimethyl xylopyranose and a trimethyl xylonic acid which underwent ready conversion to 2,3,5-trimethyl xylonolactone. This proved that the disaccharide link must have been attached to C_4, hence both xylose units must have been pyranose in character.* There being no reason to suppose that the remaining xylose units in xylan were differently constituted, the structure already referred to was adopted.

---

* **Hampton, Haworth and Hirst, *J.C.S.*, 1929, 1739.**



Trimethyl xylopyranose

2,3,5-Trimethyl xylonolactone

Hexamethyl methylxylobionate

Subsequent work has shown that the constitution of xylan is more complex than would appear from the above brief account and it will be discussed more fully later.

---

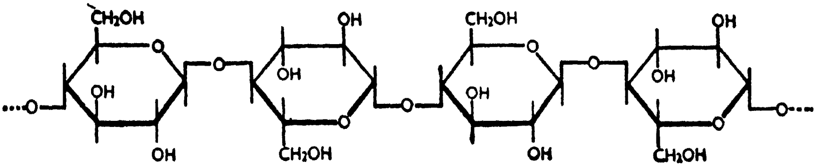
* Haworth and Percival, *J.C.S.*, 1931, 2850.

CHAPTER VII

FURTHER STUDIES ON CELLULOSE, STARCH, GLYCOGEN AND XYLAN

Cellulose

In the preceding Chapter it was explained why the fundamental constitution of cellulose could be represented as a chain of β-D-glucopyranose units linked through positions 1 and 4, and that the chain so formed certainly contained not fewer than about 200 of these residues. Such a representation, however, does not explain entirely the special properties of cellulose, in particular its relative insolubility, for it will be remembered that lichenin, which has a similar constitution, is soluble in water.



Chain of β-D-Glucopyranose Units in Cellulose

The Molecular Weight of Cellulose

The minimum molecular weight of cellulose determined by the original chain length experiment of Haworth and Machemer was of the order of about 30,000. Since the methylated cellulose used for the determination had been prepared from a cellulose acetate which had been made using sulphur dioxide and chlorine as catalysts, it was strongly suspected that degradation of the original cellulose molecule had taken place, even if it were assumed that no degradation took place during methylation. This was indeed pointed out by Haworth and emphasised on many occasions although some workers on the Continent, and in this country, failing to appreciate the reservations which Haworth put upon his findings, cast doubt on the validity of the method on the ground that certain determinations of molecular weight by physical methods indicated a much larger molecule.

One of the most readily available physical methods for determining the molecular weight of high polymers is that devised by Staudinger.* It is based on measurements of the viscosity of dilute solutions, and although essentially empirical it has been a useful guide in studies of high polymers. The method consists, briefly, in determining the specific viscosity of a dilute solution of the long chain polymer in a suitable solvent, such as *m*-cresol, as follows: The time of flow (T_1) of the pure solvent is determined, together with the time of flow (T_2) of a dilute solution of known concentration. The relative viscosity (η_r) is then T_2/T_1, and is converted into specific viscosity (η_{sp}) which equals $\eta_r - 1$, thus eliminating any contribution of the solvent to the viscosity of the solution. Staudinger's equation gives $\eta_{sp} = K_m M c$, where M is the molecular weight, *c* is the concentration in gram molecules of repeating unit per litre (the repeating unit for example in cellulose is $C_6H_{10}O_5$ and for a polyoxymethylene CH_2O) and K_m is a constant which varies from repeating unit to repeating unit; K_m for cellulose is taken to be 5×10^{-4}. Clearly the value of the method depends on the correct evaluation of the K_m constant, and this has been a matter of some difficulty for cellulose derivatives; the value being given originally as 10^{-3}. The method of determining the K_m constant is to measure the molecular weight by an independent method, such as osmotic pressure, or, if possible by a chemical method, on a series of related polymers, a so-called polyhomologous series, prepared for example by degrading cellulose acetates to different degrees.

Early values for the molecular weight of cellulose obtained by Staudinger by means of his viscosity technique, for which, incidentally, very dilute solutions must be employed in order to avoid the coalescence of individual chains into bundles, were of the order of 100,000.† More recently, values ranging from *ca* 300,000 to 800,000 have been recorded.‡ A value of 38,000 given for a specimen of cellulose acetate, which would be expected to have undergone degradation during its preparation, is in agreement with the result of the original end group assay.

Molecular weight determinations by osmotic pressure technique, which, however, demands special apparatus, are likely to be much more reliable and a figure of about 250,000 has been computed for a specimen of methylated cotton sliver.* Measurements of molecular weight by means of the ultracentrifuge have also shown that the particle size is very large. Some values are given in the accompanying table, the measurements being conducted in " cuprammonium " solution.

---

* *Die hochmolekularen organischen verbindungen*, Springer, Berlin, 1932, p. 451.

† *Trans. Faraday Soc.*, 1933, **29**, 18.

‡ See Heuser, *Cellulose Chemistry*, John Wiley & Sons, New York, 1944, p. 593.

DETERMINATIONS OF THE MOLECULAR WEIGHT OF CELLULOSE BY THE ULTRACENTRIFUGE

Material	*M.W.*	*Apparent Degree of Polymerisation*
Native Cotton†	570,000	3,500
Unbleached Cotton‡	1,500,000	9,200
Raw Georgia‡	1,000,000	6,200
Purified Linters†	150-500,000	1000-3000
Nettle Fibre‡	1,760,000	10,800
Ramie Fibre‡	1,840,000	11,300
Wood Pulp†	90-150,000	600-1000
Sulphite Pulp‡	400,000	2,900

Without discussing the relative merits of the two sets of results in the above list, it is clear that the cellulose molecule is very large. The physical methods, with the possible exception of Staudinger's viscosity method, give no information about the shape of the molecule; the high values given by the ultracentrifugal determination could be due either to the presence of very long chains or to the cohesion of shorter chains into bundles. In the viscosity determinations it is held, although direct proof is impossible to obtain, that since very dilute solutions are employed no aggregation of the chains takes place. Finally it should be made clear that all the methods of molecular weight determination, whether chemical or physical give average values only.

The Fine Structure of Cellulose

The Degradation of Cellulose during Methylation

Hess and Neumann§ recorded the observation that a specimen of cellulose methylated in an atmosphere of nitrogen, gave on hydrolysis no tetramethyl glucopyranose and was presumably to be regarded not as a terminated chain but as a looped molecule. An extensive investigation of the possibility of the incidence of degradation during methylation was conducted in the Birmingham laboratories, during the course of which specimens of methylated cellulose prepared in an atmosphere of nitrogen were also found to give no tetramethyl glucose on hydrolysis. The important observation was made, however, that the particle size of these latter specimens determined both from osmotic pressure and viscosity measurements fell progressively until a chain length of about 200 glucose units was reached.* If methylation were carried out in air, however, tetramethyl glucose appeared in the products of hydrolysis. Some of the results are collected in the following table.

---

* Haworth, Hirst, Owen, Peat and Averill, *J.C.S.*, 1939, 1885.

† Kraemer, *Ind. Eng. Chem.*, 1938, **30**, 1200.

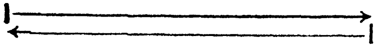
‡ Gralén and Svedberg, *Nature*, 1943, **152**, 625.

§ *Ber.*, 1937, **70**, 728.

METHYLATION EXPERIMENTS ON CELLULOSE IN NITROGEN

Material	*Number of Treatments and Temp.*	*Chain Length*		
		By viscosity	*By Osmotic Pressure*	*By End Group*
Cotton Sliver	5 at 15°	1,300	1,300	∞
Cotton Linters ,, ,,	3 at 40° 3 at 55°	600	790	∞
,, ,,	10 at 15°	340	300	∞
,, ,,	3 at 40° (in N_2) 3 at 60° (in air)	240	170	∞
,, ,,	4 at 40° (in air) 2 at 35° (in air)	170	120	176

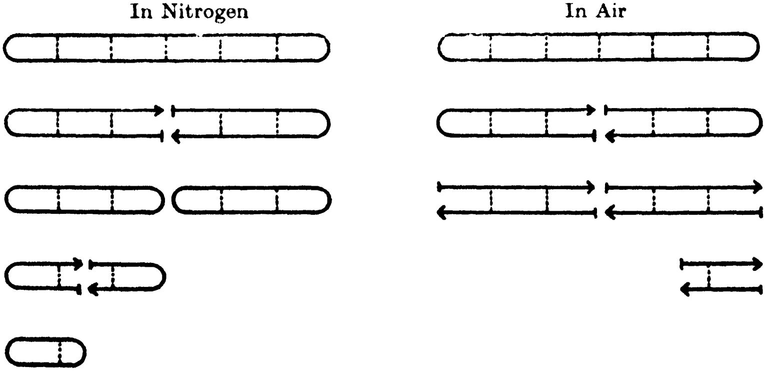
It is suggested, therefore, that the cellulose is present in the form of a large loop, which undergoes progressive shortening on methylation. In an atmosphere of nitrogen the exposed ends of the shortened loop appear to recombine, whereas in air this healing process seems to be inhibited. A possible explanation of this may be the effect of the strong alkali present on the reducing group in the presence of oxygen, for if oxidation to form a carboxylic acid takes place, recombination would be clearly impossible. It is not necessary in fact to assume that the original cellulose possesses a loop structure, although this is the view, for example, of Hess. X-ray workers have concluded from the symmetry of the X-ray diagrams that the chains of glucopyranose units are pointing in opposite directions, so that under the proper conditions, which seem to include



---

* Haworth, *J. Soc. Chem. Ind.*, 1939, 58, 917.

the absence of oxygen, head to tail combination to form a loop is therefore a possibility. Haworth has indicated the necessity for the two chains to be parallel and close together before such combination could take place, for unless this were so, under the influence of thermal movement, the chances of the reducing group forming a union with the terminal glucopyranose unit would be negligible. It is therefore suggested that cross linkages hold the two sides of the flat loop together, although the nature of the postulated cross linkages is not known. If primary valencies are involved then dimethyl glucose should arise from the points of union on the hydrolysis of methylated cellulose; the quantity, however, would be small in relation to the quantity of 2,3,6-trimethyl glucose produced. Small quantities of dimethyl glucoses have, in fact, been isolated but the possibility that these substances have arisen either through incomplete methylation or through demethylation on hydrolysis cannot be discounted. There must also be points of weakness at intervals along the chains, possibly at intervals of about 100 glucose units, although it is not known what form this weakness takes. Haworth's interpretation is depicted roughly below.

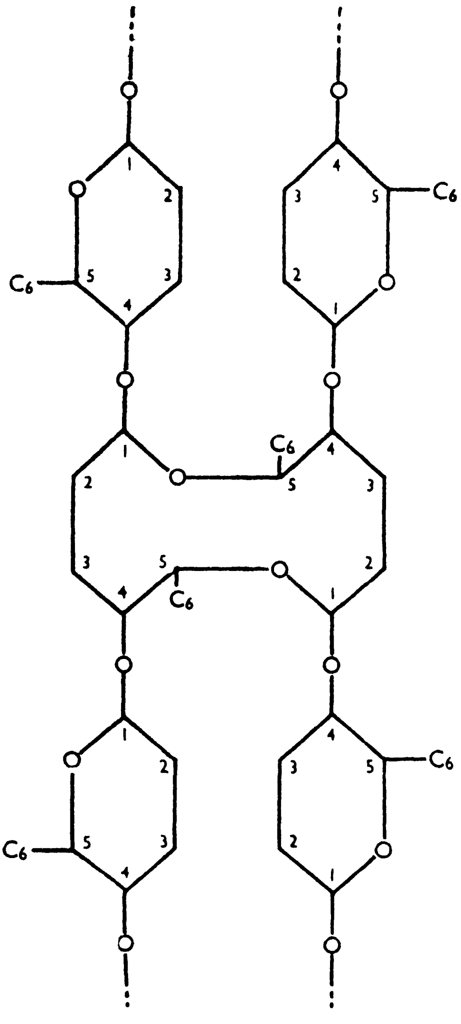


Degradation of Cellulose on Methylation

K. Hess* has also advanced a theory to explain differences between molecular weights determined by end group assay as tetramethyl glucose and by viscosity measurements. In his view cross linkages are present which he calls " Vernetzungsbrücken " or network bridges, and these are thought to be of an unusual type due to the opening of pyranose rings at some stage in the development of the cellulose. It is at least equally probable that certain of the primary

---

* *Ber.*, 1940, 73, 669.



A so-called network bridge in Cellulose after Hess

alcoholic groups are engaged in cross linkages. When the addition compound of cellobiose and potassium hydroxide, $C_{12}H_{22}O_{11}$, 2 KOH, is treated with dry methyl sulphate, methylation takes place at C_1 and C_6 and methyl cellobiosides and, after hydrolysis, 6-methyl glucose may be detected.* If, however, potassium hydroxide cellulose is used, only 2-methyl glucose derivatives can be detected on hydrolysis,* whereas potassium hydroxide-lichenin on similar treatment gave both 2-methyl and 6-methyl glucose.† It might be argued from this result that the primary alcoholic groups in cellulose are shielded in some way.

---

* Percival and Ritchie, *J.C.S.*, 1936, 1765.

Although it may be that under natural or special experimental conditions cellulose exists in the form of large loops, more commonly, terminal groups, recognisable as tetramethyl glucopyranose after methylation and hydrolysis, can be detected. This raises the question of the state of the group at the other end of the chain which might be expected to be a reducing group. Cellulose is very slightly reducing, but it appears probable that, owing to oxidation, the potential reducing group is converted into a carboxyl group. Estimations of the amount present would clearly lead to a value for the average chain length and much work has been carried out on this subject. Conductometric titrations have given a value of 96 glucose residues for cellulose from different sources,‡ although such a constant figure would seem somewhat suspicious. By titration in the presence of sodium chloride Neale§ found chain lengths in good agreement with the results of viscosity experiments, and from an examination of the ash content after the removal of the pectin, a value of 600 glucose units has been obtained|| for the chain length.

Sufficient has been said to indicate how the problem of the fine structure of cellulose has been attacked. There is, however, one further chemical method which should be mentioned, namely periodate oxidation, for it is certainly going to lead to an extension of our knowledge in this difficult field; since this reagent only attacks α-glycol residues it would be expected to give information about the postulated cross linkages or the intermeshing bridges. Already, a method of great value in the determination of the chain length of polysaccharides has been devised,¶ which depends on the production of formic acid from 1,4-linked polysaccharides by oxidation with potassium periodate, the formic acid being estimated by titration. It is from the terminal groups that the formic acid arises, one molecule being provided by the non-reducing end, and two, together with

---

* Heddle and Percival, *J.C.S.*, 1938, 1690.

† Percival and Granichstädten, *Nature*, 1942, **150**, 549.

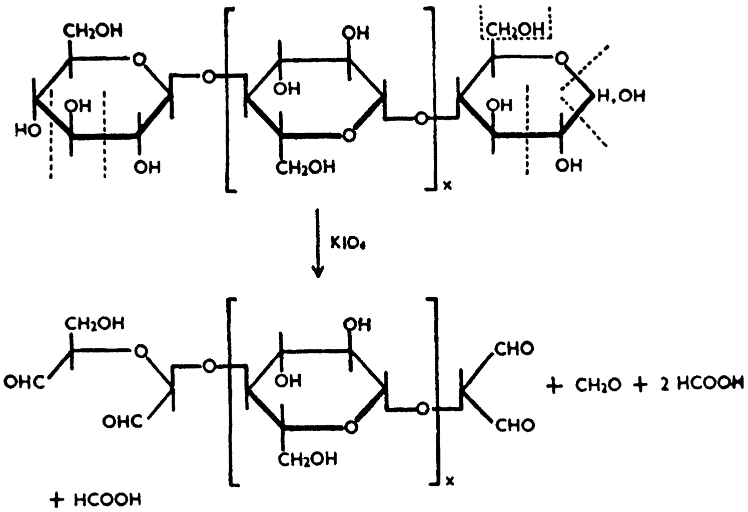
‡ E. Schmidt *et al.*, *Ber.*, 1934, **67**, 2037; 1937, **70**, 2345.

§ *Chemistry and Industry*, 1936, **55**, 602.

|| Sookne and Harris, *J. Res. Nat. Bur. Standards*, 1941, **26**, 205.

¶ Brown, Dunstan, Halsall, Hirst and J. K. N. Jones, *Nature*, 1945, **156**, 785.

a molecule of formaldehyde, from the other terminal group if we assume it is a 4-substituted glucopyranose residue. The value for



a cellulose specimen estimated by this method has been found to be at least one thousand glucose units per chain. In the above diagram it will be noted that the hydroxyl groups in the main chain of glucose units have not been represented as attacked by the potassium periodate. If the reaction is allowed to run to completion these α-glycol groups are, of course, converted to aldehyde groups.

The Effect of Substitution on the Solubility of Cellulose Derivatives

The view held by the proponents of the idea that cellulose chains are united by cross linkages involving primary or normal valency bonds, and that this explains the fact that cellulose is insoluble in ordinary solvents may be essentially correct. It is not, however, necessary to invoke such a theory to explain the phenomena of the dissolution of cellulose and its derivatives, and, indeed, if the postulated cross linkages were present at too frequent intervals along the chains, the solubility effects could not be explained at all. The aggregation of chain molecules through the van der Waals' forces and hydrogen bond formation can readily be understood if one constructs a model of cellulose from glucopyranose units linked through 1,4-β-linkages. It is possible to show that the model is almost linear and there is no difficulty in the coalescence of several such chains to form a bundle, and the X-ray work shows that this indeed occurs. The attractive forces between the chains, whether due to van der Waals' forces or to hydrogen bonds, will be approximately proportional to the length of the chains, and the longer the chains, the more force must be applied to separate them. It is to be expected also that the bundles will be stronger when all the individual chains are oriented in parallel lines. This is found to be true, for by an examination of a bundle of cellulose fibres by X-rays it is possible to determine whether the orientation is good or poor. The crystalline nature, as shown by the sharpness of the spots on the photographic plate, runs parallel to the strength of the particular fibre under examination. This is particularly easy to show for viscose rayon since the orientation of the dispersed cellulose chains can be varied according to the conditions of extrusion into the spinning bath.

When a number of parallel chains are aggregated it is clear that the relative number of available hydroxyl groups, on which the solubility naturally depends, must be diminished in comparison with a single chain of glucose units. The reactivity of the hydroxyl groups as a whole in such processes as acetylation and methylation will also be diminished, although those on the external surface will not be affected. Before the cellulose can be dissolved, therefore, it is necessary for the chains to be forced apart,* and this explains why such materials as cuprammonium hydroxide, dibenzyldiethylammonium hydroxide and phosphoric acid will dissolve cellulose, whilst sodium hydroxide, although causing swelling, will not.

The view that cellulose normally consists of chains arranged in a bundle, usually called a micelle or microfibril, also enables an explanation to be offered of some apparent anomalies in the solubilities and softening points of certain substituted cellulose derivatives. Since three hydroxyl groups are available on each glucopyranose unit, it is obvious at once that various degrees of substitution are possible. Suppose we imagine the introduction of acetyl groups to be proceeding. The solubility in water gradually increases until when the substitution is of the order of one acetyl group per glucose unit the partially acetylated cellulose so produced is soluble in water. This is at first sight rather unexpected since with the introduction of acetyl groups the substance is being made more hydrocarbon in character and hence one would expect the solubility to be diminished. This actually happens as more acetyl groups are introduced, but then the material becomes soluble in an organic solvent such as acetone. The solubility in acetone increases until the average substitution of about 2·3 hydroxyl groups in each glucose unit is reached, after which it diminishes until, with complete substitution, the product—cellulose triacetate—is completely insoluble in acetone. These results can be explained by the fact that when close packing between the individual chains is possible as in unsubstituted cellulose and in the fully substituted cellulose, the maximum attractive forces between the chains whether van der Waals' forces or hydrogen bonds, are brought into play. The introduction of substituent groups such as acetyl or methyl upsets this and the attractive forces of the solvent molecules, and thermal motion, succeed in overcoming the cohesive forces holding the chains together. The practical consequences of this effect are well known; soluble cellulose acetate for making acetate rayon contains just over two acetyl groups for each glucose unit, and the cellulose xanthate which solubilises cellulose in viscose rayon manufacture contains one xanthate group (OCSSNa) for approximately every two glucose residues.

---

* *Chemistry of Large Molecules*, 1943, edited by Burk and Grummit, Chapter 8, by E. Ott, Interscience Press, New York.

A similar effect is observed in the softening points of cellulose ethers. The softening point represents the stage at which the thermal motion begins to overcome the cohesive forces. Thus the softening temperatures of ethyl celluloses of various degrees of substitution pass through a minimum where the average degree of substitution is about two and a third. The benzyl celluloses have lower softening temperatures than the corresponding ethyl celluloses and the minimum value is found at a lower degree of substitution, namely at about two benzyl residues per glucose unit. This is in agreement with the theory, for the larger benzyl groups would be expected to cause greater disturbances in the packing of the chains.*

It must not be supposed, however, that natural celluloses, even cotton cellulose, are composed entirely of well oriented chains. This state of affairs exists only in the crystalline regions in which the chains of glucose units are oriented in opposite directions. There are however portions of the fibre which give no sharp X-ray diagram, and in this amorphous cellulose there is random orientation which results in a higher degree of chemical reactivity, and more rapid penetration by dyestuff solutions.

---

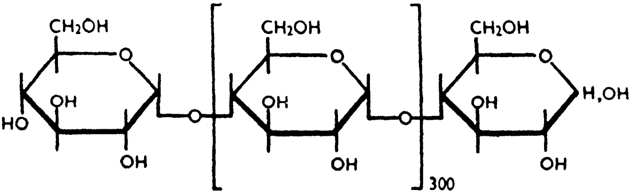
* Lorand, *Ind. Eng. Chem.*, 1938, **30**, 527.

The Starch Polysaccharides: Amylose and Amylopectin

Amylose

Owing to the difficulty of separating pure amylose from the starch grain, our knowledge of the constitution of this portion of the starch molecule has been, until quite recently, inexact. There is little doubt that the discovery by C. S. Hanes that it is possible to prepare by the use of enzymes, a " synthetic " amylose gave an impetus to investigations on this subject.

As mentioned in the previous chapter, the amylose fraction of starch which recently has been obtained crystalline, is completely hydrolysed to maltose by β-amylase, and although the amount in most starches is only of the order of 20% it is this amylose constituent which gives the intense blue colour with iodine. The present view of its constitution is that it consists of very long chains of α-D-glucopyranose units linked as in maltose; the chains are estimated to contain about 300 glucose units, from the yield of tetramethyl glucopyranose (0·3%) obtained from the hydrolysis of methylated amylose, and this corresponds to a molecular weight of about 50,000.[*] Measurements of reducing power by colorimetric estimation of the reduction of silver oxide, carried out by Meyer, gave a value corresponding to about 1/200th of the value for glucose,[*] a result in reasonable agreement with that obtained by the previous method. In another series of measurements which gives results in agreement with the foregoing, the chain length is determined by a colorimetric method in which 3,5-dinitrosalicylic acid in sodium hydroxide is used. The intensity of colour is referred to standards made up with maltose.[†] The specific rotation of amylose treated with alkali and then acidified was found to be +220°±5° and the calculated value,



Amylose

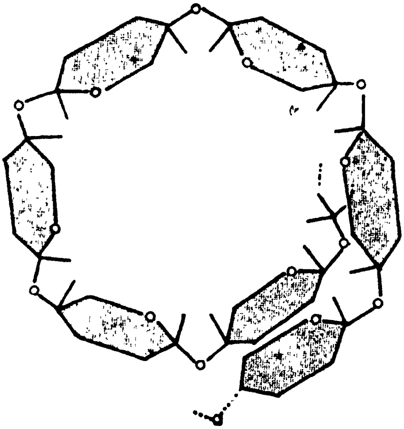
---

[*] Meyer, Wertheim and Bernfeld, *Helv. Chim. Acta*, 1940, **23**, 864; 1941, **24**, 378; Hess and Krajnc, *Ber.*, 1940, **73**, 976; Hassid and McCready, *J. Amer. Chem. Soc.*, 1943, **65**, 1157.

[†] Meyer, Noelting and Bernfeld, *Helv. Chim. Acta*, 1948, **31**, 103.

on the assumption that all the glucose residues are joined by α-links as in maltose, is +230°; this fits in with the quantitative yield of maltose produced on hydrolysis with β-amylase. These results all agree with the hypothesis that amylose is a long chain molecule; a practical demonstration of this is found in the formation of strong films and fibres of amylose acetate, similar to those given by cellulose acetate. Amylose can also give an X-ray pattern of the so-called V type similar to that given by cellulose.

The suggestion has been made that the glucose units in amylose are not arranged in the straight chain form characteristic of cellulose, but are coiled in a helical fashion,[*] with six pyranose rings forming each turn of the helix. Such an arrangement is possible in this case with the α-linkages, although it would be quite impossible for cellulose because in that case the adjacent glucopyranose rings have to be rotated through 180° and hence any deviation from a straight line in cellulose is automatically cancelled out. Investigations[†] with X-rays on crystalline amylose and on the amylose iodine complex support this helical structure. In the stretched amylose acetate fibres it is considered likely that the spirals are pulled out like an extended spring.



Schematic Representation of Helical Amylose Structure

Freudenberg has used the idea of the helical structure to explain the characteristic colour of the amylose iodine complex, for the inner dimensions of the spiral appear to be such that an iodine molecule

---

[*] Hanes, *New Phytologist*, 1937, **36**, 189; Freudenberg *et al.*, *Naturwissenschaften*, 1939, **27**, 850.

[†] Rundle and Edwards, *J. Amer. Chem. Soc.*, 1943, **65**, 2200; Rundle and French, *ibid.*, 1707.

(6·3Å × 3·8Å) can be deposited inside. *The hydrogen atoms at* C_1 and C_4, that is those on either side of the glycosidic bridges, are presumed to form a hydrocarbon lining within the spiral measuring about 5Å across and one iodine molecule is said to be associated with ten of these hydrogen atoms, and since iodine gives blue solutions in hydrocarbons, this is held to explain the phenomenon. It should be pointed out, however, that Freudenberg's model is based on the assumption of a *cis* strainless form for the glucopyranose rings and may not be correct in all details. X-ray studies by Rundle and his co-workers* give general support to this view, and their observation that the amylose iodine complex exhibits dichroism of flow, the absorption of light being strongest along the flow lines and least at right angles to them,† is cited as evidence that the iodine molecules are arranged with their long axes parallel to the long axis of the starch iodine complex. Objections to this interpretation of the cause of the blue colour have been raised by Meyer and Bernfeld‡ on the ground that substances known definitely not to have a spiral arrangement of units such as methylated cellulose, cellulose swollen by zinc chloride and even inorganic compounds such as praeseodymium and lanthanum salts give blue colours with iodine similar to those given by starch. In the view of the Geneva workers the blue colour is due to colloidal precipitates with greatly distorted lattices containing fissures in which the iodine molecules are deposited. Since in starch, association in solution to form micelles is greatest when the tendency to crystallise is greatest, in this case when the chains are longest, it is considered that this explains why amylose but neither amylopectin nor short chain dextrins give the blue colour with iodine. The reader will observe however that these arguments of Meyer do not really dispose of the theory of the dependence of the blue colour on a helical structure; in the place of the hypothetical fissures of Meyer a perfectly possible hollow cylinder is substituted, and the behaviour of inorganic precipitates does not disprove the proposed explanation in this particular instance.

The Synthesis of Amylose by Enzymes

Biochemical researches on the breakdown of glycogen by the phosphorylases found in animal tissues such as muscles and liver, resulted

---

* Rundle and Edwards, *J. Amer. Chem. Soc.*, 1943, **65**, 2200; Rundle and French, *ibid.*, 1707.

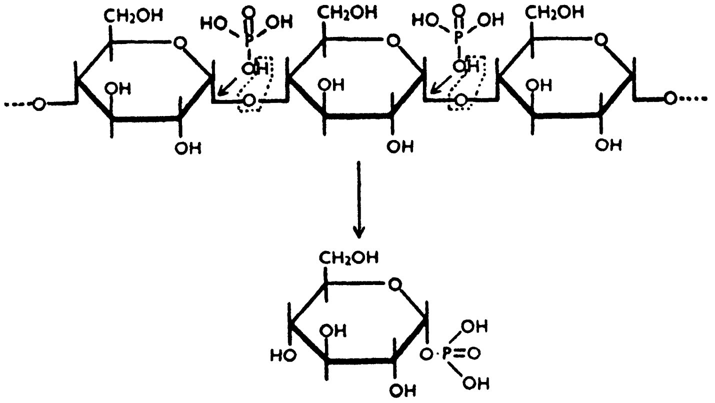
† Rundle and Baldwin, *J. Amer. Chem. Soc.*, 1943, **65**, 554.

‡ *Helv. Chim. Acta*, 1941, **24**, 389.

in the demonstration by C. F. Cori and G. T. Cori in 1936 that glycogen underwent conversion to glucose 1-phosphate, the so-called Cori ester. It was also demonstrated that the reaction was reversible and that polysaccharides could be synthesised from glucose 1-phosphate, inorganic phosphate and the phosphorylases.[*]

Cori and his co-workers suggested that the glucose phosphate involved was α-D-glucopyranose 1-phosphate[†] formed by a process of phosphorolysis, as distinct from hydrolysis with water, the inorganic phosphate entering at the glycosidic linkages.

Phosphorolysis of Amylose



α-D-Glucopyranose 1-phosphate

Wolfrom and co-workers[‡] synthesised both the α- and β-forms of D-glucopyranose 1-phosphate from acetobromoglucose and silver phosphate and showed the α-form to be identical with the Cori ester.

Starch chemistry received a great impetus from the important related discovery of C. S. Hanes[§] that the degradation of potato starch by either potato or pea phosphorylase could be reversed, at least partially. When glucose 1-phosphate was incubated with potato phosphorylase a product, closely resembling starch granules in appearance, was produced, this gave a deep blue colour with iodine, was converted completely into maltose by the agency of β-amylase, and gave an X-ray pattern resembling that of starch.[||] Investigations on this material by the methylation technique[*] showed that the chain of glucopyranose units had a minimum length of 80-90, in agreement with that of 100 deduced by Hanes from the value of the copper number, a determination which depends on the reducing power. Calculations from viscosity measurements with the Staudinger equation gave a chain length of 80-160 glucose units.

---

[*] Cori, Cori and G. Schmidt, *J. Biol. Chem.*, 1939, **129**, 629; Cori, G. Schmidt and Cori, *Science*, 1939, **89**, 464; Kiessling, *Naturwiss*, 1939, **27**, 129.

[†] Cori, Colowick and Cori, *J. Biol. Chem.*, 1937, **121**, 465.

[‡] *J. Amer. Chem. Soc.*, 1941, **63**, 1050.

[§] *Proc. Roy. Soc.*, 1940, B., **128**, 421; **129**, 174.

[||] Astbury, F. O. Bell and Hanes, *Nature*, 1940, **146**, 558.

A somewhat different result was obtained by Hassid and McCready[†] from the gravimetric assay method, since no 2,3,4,6-tetramethyl glucose could be detected when a fully methylated specimen of the " synthetic starch " was hydrolysed, the only recognisable product being 2,3,6-trimethyl glucose. These authors concluded either that the chains were very long or that they existed in the form of continuous loops. The viscosity method gave values for the molecular weight of 85,000 for the acetate and 54,000 for the methylated product. These figures would correspond to a molecule containing about 300 glucose units.

When glucose 1-phosphate is treated with muscle phosphorylase the product isolated is not glycogen, but a polysaccharide which gives a deep blue colour with iodine and is completely hydrolysed by β-amylase. This on examination by the end group assay process was shown to consist of a chain of 200 glucose units.[‡] From the results it is evident that products which are indistinguishable from amylose can be prepared from glucose 1-phosphate and phosphorylases, although there may be some variations of chain length depending on the particular experimental conditions employed.

$$\text{Glucose 1-phosphate} \quad \underset{\longleftarrow}{\overset{\longrightarrow}{\textit{phosphorylase}}} \quad \text{amylose} + \text{inorganic phosphate}$$

The process is a true reversible reaction because the ratio of inorganic phosphate to ester phosphate reaches the same value when the equilibrium is approached from either direction for wide variations of concentrations of the amylose and the total phosphate.

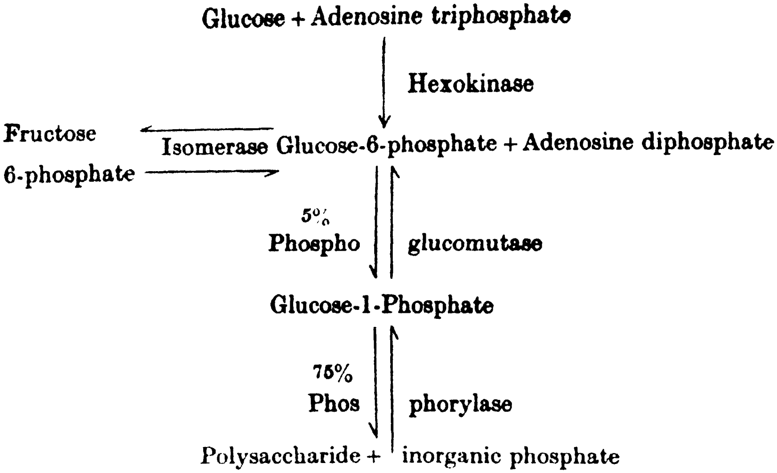
One important feature of the process is that purified potato phosphorylase from which all dextrins have been removed, and pure glucose 1-phosphate, fail to give synthetic starch unless a " primer " is added. The priming agent may be starch, starch dextrins or glycogen although the product formed is independent of the particular priming agent. Thus the addition of glycogen produces the same synthetic amylose as when plant starch is used, but the synthetic amylose itself will not act as a priming agent. By the use of several enzyme systems the production of a polysaccharide from glucose *in vitro* has been achieved.* When, to a mixture of phosphoglucomutase phosphorylase and hexokinase, glucose and adenosine triphosphate were added a synthetic polysaccharide was produced according to the following scheme.

---

[*] Haworth, Heath and Peat, *J.C.S.*, 1942, 55.

[†] *J. Amer. Chem. Soc.*, 1941, **63**, 2171.

[‡] Hassid, G. T. Cori and McCready, *J. Biol. Chem.*, 1943, **148**, 89.


Glucose + Adenosine triphosphate
Hexokinase
Fructose 6-phosphate
Isomerase
Glucose-6-phosphate + Adenosine diphosphate
5%
Phospho
glucomutase
Glucose-1-Phosphate
75%
Phos
phorylase
Polysaccharide + inorganic phosphate

The " energy rich " phosphate bond in the adenosine triphosphate supplies the energy necessary for the conversion of glucose to glucose 6-phosphate. Little change in free energy is required for the subsequent transformations. It may be noted that α-L-glucose-1-phosphate is not converted into a polysaccharide by potato phosphorylase.†

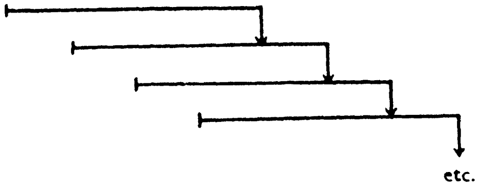
Amylopectin

The major constituent of the starch grain, amylopectin, responsible for the formation of starch pastes, is more complex than amylose. In the preceding chapter the results of many estimations of chain length on various starches were summarised and found to give the same result of about 24 glucose units. It was well known from the results of physical measurements that starch was a much larger molecule than this would appear to indicate, and early attempts were made to explain the discrepancy by assuming a physical aggregation of short chains, on the ground that the model of the starch molecule with its 1,4-α-links, unlike cellulose with its 1,4-β-links, could easily lend itself to a zig-zag or a spiral arrangement, which might, by physical interlocking cause aggregation to a large molecule. Various reasons including almost complete absence of reducing power soon made it evident that such an explanation was inadequate. For a short time it was thought that association by van der Waals' forces and hydrogen bonds might explain the phenomenon. It has been pointed out however* that processes such as methylation would be expected to diminish these effects by the substitution of the hydoxyl groups, but methylated and acetylated starches still have very high molecular weights. To overcome the difficulty of accommodating the chain of 24 glucose units into a large molecule with a particle weight of at least 5×10^5, and to explain why dimethyl glucoses were always given as well as tetramethyl glucose (4·5%) and 2,3,6-trimethyl glucose (*ca* 90%) when methylated starches were hydrolysed, the suggestion was made† that primary valencies unite the relatively short chain " repeating units " to form a large aggregate or " laminated " structure. A union of this type had in fact been proposed previously for xylan to explain the occurrence of a monomethyl xylose in the products of hydrolysis of methylated xylan. Each repeating unit was presumed to be united to the next by a normal glycosidic linkage, operating from the potentially reducing end of the chain, and joined to the neighbouring chain through one of the available primary alcoholic groups. This could be expressed diagrammatically thus:

---

* Colowick and Sutherland, *J. Biol. Chem.*, 1943, **144**, 423.

† Hassid, Doudoroff and Barker, *J. Amer. Chem. Soc.*, 1944, **66**, 1416.



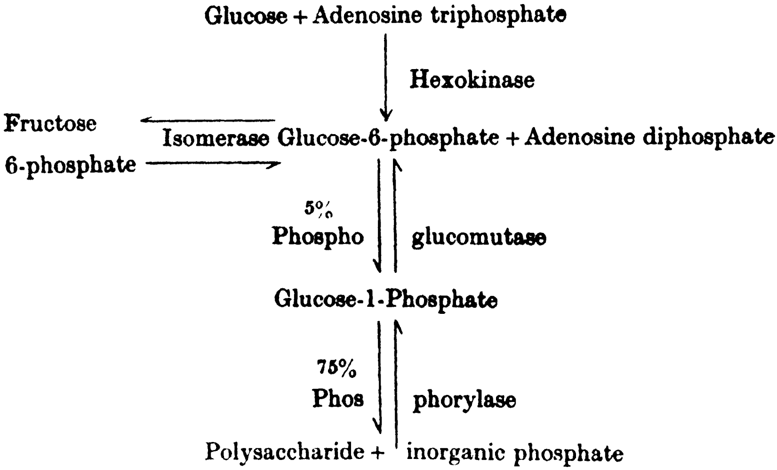
The expression " laminated " as used here should not be associated with the precise regularity found in a laminated sheet of plywood, since we are concerned with the union of a series of chains in three dimensions and not with a series of parallel plates. The term ramified might be more appropriate.

Evidence supporting this view has been provided by experiments ticular priming agent. Thus the addition of glycogen produces the same synthetic amylose as when plant starch is used, but the synthetic amylose itself will not act as a priming agent. By the use of several enzyme systems the production of a polysaccharide from glucose *in vitro* has been achieved.* When, to a mixture of phosphoglucomutase phosphorylase and hexokinase, glucose and adenosine triphosphate were added a synthetic polysaccharide was produced according to the following scheme.

---

* Bawn, Hirst and G. T. Young, *Trans. Faraday Soc.*, 1940, **36**, 880.

† Haworth, Hirst and Isherwood, *J.C.S.*, 1937, 577.


Glucose + Adenosine triphosphate
Hexokinase
Fructose
6-phosphate
Isomerase
Glucose-6-phosphate + Adenosine diphosphate
5%
Phospho
glucomutase
Glucose-1-Phosphate
75%
Phos
phorylase
Polysaccharide + inorganic phosphate

The " energy rich " phosphate bond in the adenosine triphosphate supplies the energy necessary for the conversion of glucose to glucose 6-phosphate. Little change in free energy is required for the subsequent transformations. It may be noted that α-L-glucose-1-phosphate is not converted into a polysaccharide by potato phosphorylase.†

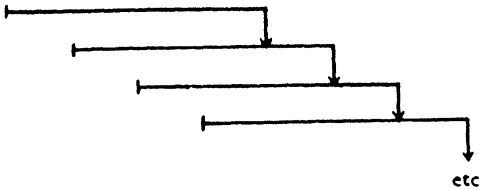
Amylopectin

The major constituent of the starch grain, amylopectin, responsible for the formation of starch pastes, is more complex than amylose. In the preceding chapter the results of many estimations of chain length on various starches were summarised and found to give the same result of about 24 glucose units. It was well known from the results of physical measurements that starch was a much larger molecule than this would appear to indicate, and early attempts were made to explain the discrepancy by assuming a physical aggregation of short chains, on the ground that the model of the starch molecule with its 1,4-α-links, unlike cellulose with its 1,4-β-links, could easily lend itself to a zig-zag or a spiral arrangement, which might, by physical interlocking cause aggregation to a large molecule. Various reasons including almost complete absence of reducing power soon made it evident that such an explanation was inadequate. For a short time it was thought that association by van der Waals' forces and hydrogen bonds might explain the phenomenon. It has been pointed out however* that processes such as methylation would be expected to diminish these effects by the substitution of the hydoxyl groups, but methylated and acetylated starches still have very high molecular weights. To overcome the difficulty of accommodating the chain of 24 glucose units into a large molecule with a particle weight of at least 5×10^5, and to explain why dimethyl glucoses were always given as well as tetramethyl glucose (4·5%) and 2,3,6-trimethyl glucose (*ca* 90%) when methylated starches were hydrolysed, the suggestion was made† that primary valencies unite the relatively short chain " repeating units " to form a large aggregate or " laminated " structure. A union of this type had in fact been proposed previously for xylan to explain the occurrence of a monomethyl xylose in the products of hydrolysis of methylated xylan. Each repeating unit was presumed to be united to the next by a normal glycosidic linkage, operating from the potentially reducing end of the chain, and joined to the neighbouring chain through one of the available primary alcoholic groups. This could be expressed diagrammatically thus:

---

* Colowick and Sutherland, *J. Biol. Chem.*, 1943, **144**, 423.

† Hassid, Doudoroff and Barker, *J. Amer. Chem. Soc.*, 1944, **66**, 1416.



The expression " laminated " as used here should not be associated with the precise regularity found in a laminated sheet of plywood, since we are concerned with the union of a series of chains in three dimensions and not with a series of parallel plates. The term ramified might be more appropriate.

Evidence supporting this view has been provided by experiments on rice starch.[*] In the first place it was shown that the methylation of starch either in nitrogen or in the presence of oxygen did not cause the curious degradation which has been reported for cellulose (see page 126), and methylated starches with molecular weights of the order of 5×10^5 giving viscous solutions could be prepared. When such a product was heated with a solution of oxalic acid in aqueous methanol, and the process interrupted at intervals, a series of fractions was obtained and purified by precipitation from boiling water. These disaggregated products were then remethylated to substitute any hydroxyl groups exposed in disaggregation during which the viscosity, and hence the particle weight diminished regularly. The original fraction of methylated rice starch employed had a molecular weight of 525,000 and a repeating unit of 6,100 (30 glucose residues) so that in the original material more than 2500 methylated glucose residues were present in 80-90 repeating units. After the disaggregation process had continued for two hundred and twelve hours, a product was obtained with a molecular weight of about 20,000, determined by measurements of viscosity, osmotic pressure and sedimentation equilibrium by the ultracentrifuge. A determination of the proportions of methylated glucoses liberated on hydrolysis gave 2,3,4,6-tetramethyl methylglucoside (3·5%), 2,3,6-trimethyl methylglucosides (92%) and dimethyl methylglucosides (1·9%), the first value corresponding to a repeating unit of 31 glucose residues. This result may be compared with the value for a fraction of the methylated rice starch (see page 116) prepared by methylation in an atmosphere of nitrogen which gave 3·3%, 88% and 3·4% yields of the above products respectively, corresponding to a chain length of 30 glucose units.

---

* Bawn, Hirst and G. T. Young, *Trans. Faraday Soc.*, 1940, **36**, 880.

† Haworth, Hirst and Isherwood, *J.C.S.*, 1937, 577.

The disaggregated methylated rice starch was much more soluble in organic solvents and in cold water than the original material, while the specific rotation, $[\alpha]_D + 209°$, was virtually unchanged from that of the starting material ($+205°$). If degradation to dextrins had occurred by the rupture of the maltose links then the rotation would have fallen considerably. It was considered therefore that " the disaggregation reaction has not involved any breakdown in the repeating units but is concerned with the separation intact of the repeating units from larger aggregates of these units".[*] The molecular weight (20,000) of the smallest macromolecule obtained corresponds to an aggregation of three (or possibly four) of these repeating units. It was not found possible to proceed beyond this point without causing breakdown within the repeating units themselves. Since the smallest aggregate actually isolated contains about 100 glucose units, this result is also open to the interpretation, that amylopectin is made up of aggregates of this larger repeating unit, a possibility which Hirst and his co-workers recognised. The particular point to note however, is that the average length of chain remained constant during the disaggregation process, whilst the " physical " molecule gradually diminished in size.

---

[*] Hirst and G. T. Young, *J.C.S.*, 1939, 951, 1471.

More recent work on the chain length of various starches, by the potassium periodate oxidation technique* has shown that the chain length of the repeating unit of the amylopectin portion of most starches is about 20 units instead of the figure of 24-30 arrived at by the methylation method. These new results have been reached by estimating the amylose content by potentiometric titration with iodine and correcting the chain length, calculated from the liberation of formic acid from the non-reducing end group (see page 130), accordingly. As examples may be quoted: potato (24), banana (21), tapioca (20), waxy maize (which contains no amylose) (20), rice (20) amylopectins, although exceptions are found in arrowroot (25) and sweet potato (26) amylopectins. These lower values are due, of course, to the fact that the earlier chain length experiments were carried out on whole starches and that amylopectin preparations entirely free from amylose have not been available in sufficient quantity for chain length measurements by the methylation method.

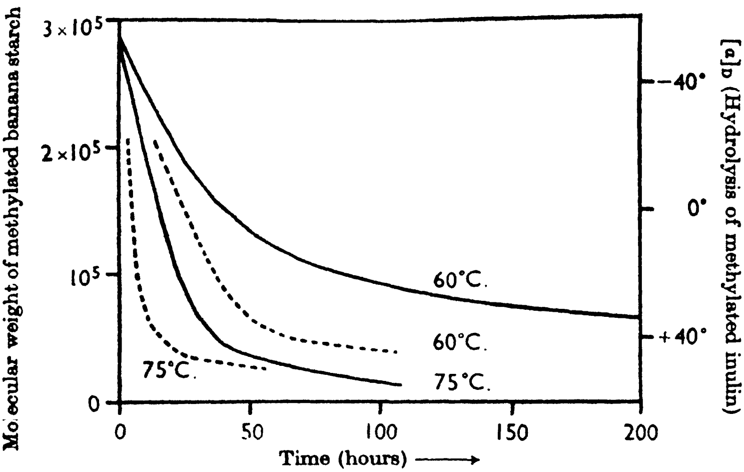
The kinetics of the disaggregation process of methylated chestnut starch have been studied with very important results.† By isolating fractions at different intervals of time in the disaggregation process, remethylating and determining molecular weight by osmotic pressure and viscosity measurements, and plotting the results against time, the rate of the disaggregation process could be followed. It was calculated to be six or seven times slower than the hydrolysis of methylated inulin and with an activation energy of 25,000 cals. Reference to the structure of inulin (page 119) will show that the hydrolysis must proceed by the rupture of glycosidic links involving primary valencies, for there is no possibility of aggregation since the physical molecular weight is in agreement with the value found by end group assay. The energy of activation is also much too high to be accounted for in overcoming either van der Waals' forces or hydrogen bonds (5000-8000 cals.).

---

* Brown, Halsall, Hirst and J. K. N. Jones, *J.C.S.*, 1948, 27.

† Bawn, Hirst and Young, *Trans. Faraday Soc.*, 1940, **36**, 880.

Methylated banana starch has also been studied by the disaggregation method* and the accompanying diagram shows how the disaggregation process and the hydrolysis of methylated inulin compare. The results obtained for banana starch were very similar to those described for rice starch.



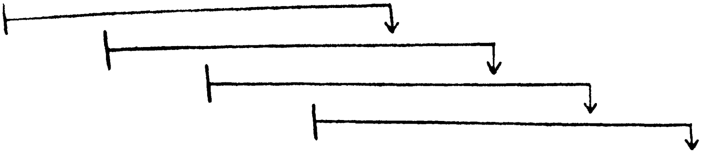
Action of 1% oxalic acid in aqueous methanol at 60° and 75°
on methylated banana starch ————
on methylated inulin - - - - - - - - - -

In passing it should be stated that within the starch granule hydrogen bonding perhaps may play a part, since this would explain why the granules undergo disruption with acids, but a physical aggregation theory to explain the high molecular weight would also demand the presence of such bonds in solution and this cannot be so from the evidence presented.

The next question was to decide the point of union between one repeating unit and its neighbour. It will have been noticed that the proportion of dimethyl methylglucosides (3·3%) obtained from the hydrolysis of the methylated rice starch was considerably greater than that (1·9%) obtained from the remethylated disaggregated material. This is in agreement with theory. If there are four repeating units in the disaggregated product there will be four parts of tetramethyl glucose to three of dimethyl glucose, whereas if there

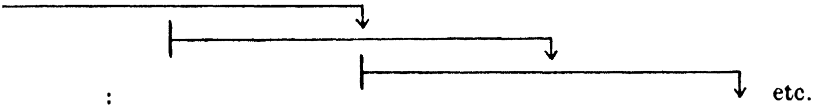
---

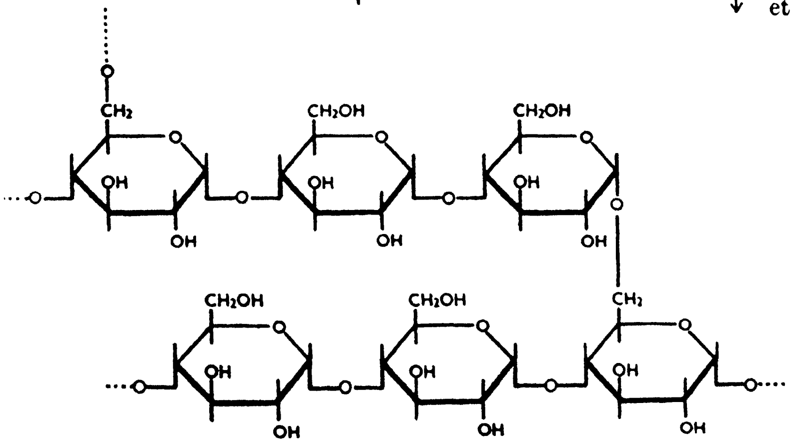
* Hawkins, Jones and Young, *J.C.S.*, 1940, 390.



are, say, 125 repeating units in the giant molecule, the ratio of dimethyl glucose to tetramethyl glucose will be practically unity (124/125).

Evidence is forthcoming that the dimethyl glucose at the junction is 2,3-dimethyl glucose.* Since it formed both methylfuranosides and methylpyranosides, the hydroxyls on C_4 and C_5 are unsubstituted; the amide of the corresponding dimethyl gluconic acid gave a negative Weerman test so that a methoxyl group is present on C_2, and the monotosyl derivative reacted with sodium iodide in acetone indicating that the hydroxyl group on C_6 was free. This result makes it evident that the reducing group in one chain is joined by a glycosidic link to *one* of the primary alcoholic groups on the neighbouring chain, and a similar conclusion has been reached by other workers.†





The Cross Linking of Chains in Amylopectin

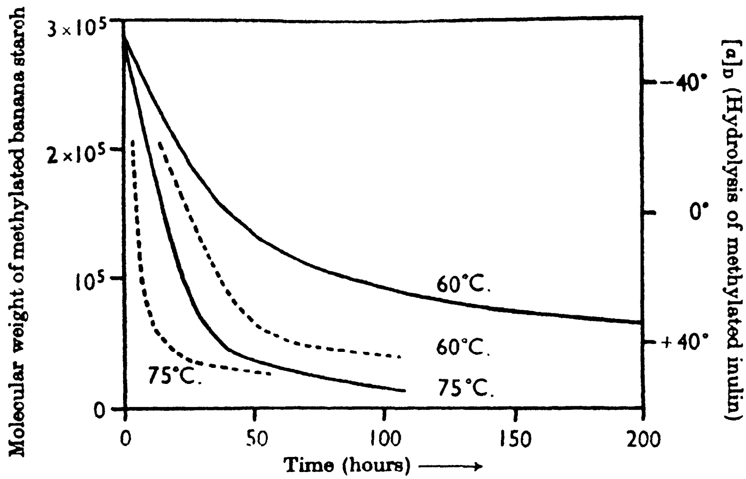
The possibility exists that not all the branchings are due to 1,6-links, and experimental proof of this has been supplied by Hirst and to be accounted for in overcoming either van der Waals' forces or hydrogen bonds (5000-8000 cals.).

---

* Barker, Hirst and Young, *Nature*, 1941, **147**, 296.

† Freudenberg, *Naturwiss*, 1939, **27**, 850; *ibid.*, 1940, **28**, 264; Freudenberg and Boppel, *Ber.*, 1940, **73**, 609.

Methylated banana starch has also been studied by the disaggregation method* and the accompanying diagram shows how the disaggregation process and the hydrolysis of methylated inulin compare. The results obtained for banana starch were very similar to those described for rice starch.



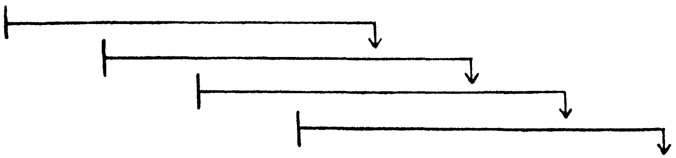
Action of 1% oxalic acid in aqueous methanol at 60° and 75°
on methylated banana starch ————
on methylated inulin - - - - - - - - - - -

In passing it should be stated that within the starch granule hydrogen bonding perhaps may play a part, since this would explain why the granules undergo disruption with acids, but a physical aggregation theory to explain the high molecular weight would also demand the presence of such bonds in solution and this cannot be so from the evidence presented.

The next question was to decide the point of union between one repeating unit and its neighbour. It will have been noticed that the proportion of dimethyl methylglucosides (3·3%) obtained from the hydrolysis of the methylated rice starch was considerably greater than that (1·9%) obtained from the remethylated disaggregated material. This is in agreement with theory. If there are four repeating units in the disaggregated product there will be four parts of tetramethyl glucose to three of dimethyl glucose, whereas if there

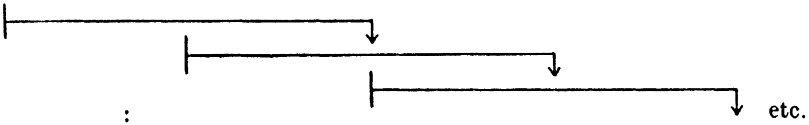
---

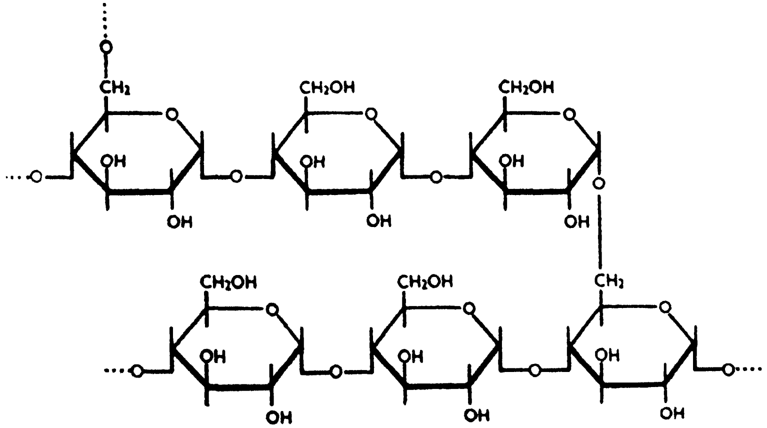
* Hawkins, Jones and Young, *J.C.S.*, 1940, 390.



are, say, 125 repeating units in the giant molecule, the ratio of dimethyl glucose to tetramethyl glucose will be practically unity (124/125).

Evidence is forthcoming that the dimethyl glucose at the junction is 2,3-dimethyl glucose.[*] Since it formed both methylfuranosides and methylpyranosides, the hydroxyls on C_4 and C_5 are unsubstituted; the amide of the corresponding dimethyl gluconic acid gave a negative Weerman test so that a methoxyl group is present on C_2, and the monotosyl derivative reacted with sodium iodide in acetone indicating that the hydroxyl group on C_6 was free. This result makes it evident that the reducing group in one chain is joined by a glycosidic link to *one* of the primary alcoholic groups on the neighbouring chain, and a similar conclusion has been reached by other workers.[†]





The Cross Linking of Chains in Amylopectin

The possibility exists that not all the branchings are due to 1,6-links, and experimental proof of this has been supplied by Hirst and his co-workers[*] as a result of oxidation experiments with periodic acid. This reagent attacks α-glycol groups as pointed out previously, and the glucose residues at the branching points, if 1,6-linkages are involved, must have the hydroxyl groups on C_2 and C_3 free, and must therefore be oxidised to a dialdehyde by periodic acid. If, therefore, all the links responsible for the branching in amylopectin were of the 1,6-type, oxidation with periodic acid followed by hydrolysis should give no free glucose; every glucopyranose ring in the giant molecule would be broken to form a dialdehyde. If, however, in some cases the branching linkage involved either C_2 or C_3, then in these cases no α-glycol group would remain for attack, and the particular unit concerned should not then be attacked by periodic acid, and would appear as glucose on hydrolysis after complete oxidation with periodate. By the use of the paper chromatogram (see page 168) the hydrolysis products of such oxidised starches from the potato, acorn, sago and waxy maize and the limit dextrin (see page 145) (obtained from waxy maize by the action of purified β-amylase), have all been shown to give small amounts of glucose, amounting to less than 1% of the weight of starch used. This proves that more than 75% of the branched links are 1,6, but that a small proportion probably involve the hydroxyl groups on C_2 and C_3.

---

[*] Barker, Hirst and Young, *Nature*, 1941, **147**, 296.

[†] Freudenberg, *Naturwiss*, 1939, **27**, 850; *ibid*., 1940, **28**, 264; Freudenberg and Boppel, *Ber*., 1940, **73**, 609.

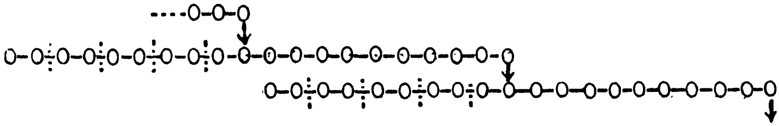
The " laminated structure "[†] explains the known facts although chemical investigations have not yet been able to show at what point in the chain the branching is to be found. The results of enzymic hydrolysis *appear* to give an answer to this question. When potato or maize starch is treated with β-amylase, hydrolysis takes place to give maltose, and a dextrin which is found by end group assay[‡] to consist of a chain of 11-12 glucose units. The β-amylase is only capable of hydrolysing 1,4-α-links with the removal of maltose. When the 1,6-link is reached the amylolysis ceases. Since this residual dextrin has an end group it follows that the enzyme hydrolysis must stop before the polymeric 1,6-link, or branch, is reached and this state of affairs may be represented diagrammatically below. It would *seem* therefore that the arrowheads representing the polymeric bonds are about half way along the chain of glucose units. Since the end group determinations can only represent an average, this interpretation of the result is open to question, for the polymeric links could be distributed at random to give the same result.

---

[*] Halsall, Hirst, J. K. N. Jones and Roudier, *Nature*, 1947, **160**, 899.

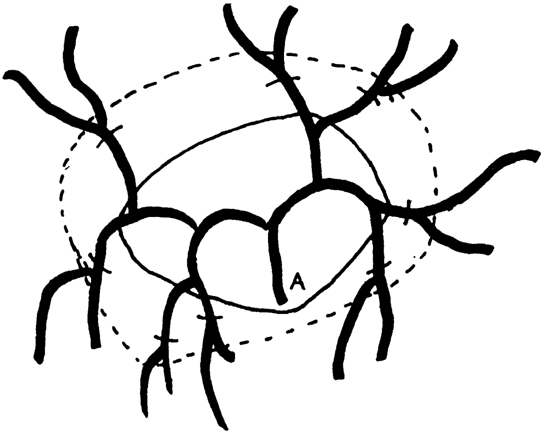
[†] Haworth, *J.C.S.*, 1946, 543; *Nature*, 1947, **160**, 901.

[‡] Haworth, Kitchen and Peat, *J.C.S.*, 1943, 619; Meyer and Bernfeld, *Helv. Chim. Acta*, 1940, **23**, 875.



K. H. Meyer* has proposed a scheme of multiple branching to explain the results he obtained by enzymolysis. With β-amylase, maize amylopectin yielded a residual dextrin (Grenzdextrin I) in a yield of about 45%, which on methylation and hydrolysis gave 9% of tetramethyl glucose corresponding to a chain of 12 glucose units. Treatment with α-glucosidase (maltase) from yeast split off glucose to give " Grenzdextrin II ". This is taken as evidence that the 1,6-linkage which is broken is an α-link. β-amylase can then attack the chain again with further degradation to maltose and " Grenzdextrin III "*, amounting to 55% of dextrin I. From results of this kind a multiple branched model has been constructed.

Simplified Representation of Amylopectin (Meyer)



A = Reducing Group

- - - - = Limit of action of β-amylase (I)

✚ = Limit of action of α-glucosidase (II)

—— = Limit of action of β-amylase in second treatment (III)

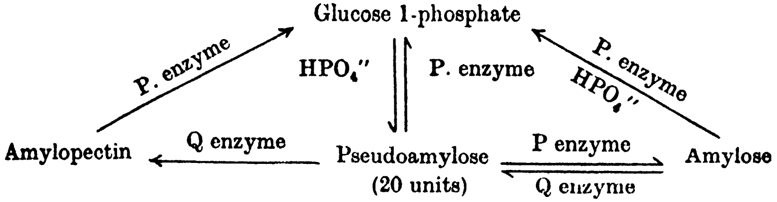
* *Advances in Colloid Science*, 1942, **1**, 143, Interscience Publishers Inc., New York; *Nature*, 1947, **160**, 900.

A model of this kind may explain the enzymic hydrolyses and it must be admitted that the results of the end group assay experiments do not exclude the possibility of multiple branching since the method depends on the relative proportions of tetramethyl to trimethyl glucose and the presence of the appropriate number of short and long chains would give the same result. The results of the disaggregation experiments, however, can hardly be explained by such a model and the adoption of the laminated structure at any rate as a basis for future research would seem reasonable.

The Synthesis of Amylopectin by Enzymes

Since the action of potato and pea phosphorylase (P enzyme) was instrumental in synthesising amylose from glucose l-phosphate, a search has been made for the conditions under which amylopectin could be built up. As a result of this, Haworth, Peat and Bourne* have isolated an enzyme from potato juice which they have named Q enzyme. This substance was found to act on amylose without the production of a reducing sugar, but the reaction of the product to iodine was changed, a red instead of a blue colour resulting. The Q factor did not show any synthetic activity with glucose l-phosphate unless the potato phosphorylase or P enzyme was present. In these circumstances a polysaccharide was produced which was almost certainly amylopectin, gave a red colour with iodine, underwent conversion to maltose by β-amylase only to the extent of 46% and had a chain length of 20 glucose units.†

The explanation advanced is as follows: Glucose l-phosphate is built up into a substance called pseudoamylose, containing an unbranched chain of 20 glucose units, and in the absence of the Q factor this is converted into the long chain synthetic amylose of Hanes. The Q enzyme, however, causes the building up from the pseudoamylose of the laminated arrangement characteristic of amylopectin.



---

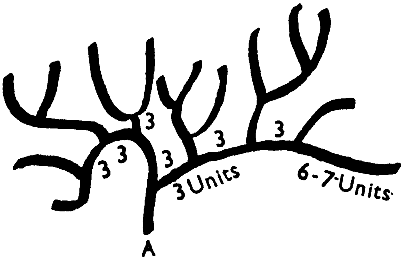
* *Nature*, 1944, **154**, 236.

† Bourne and Peat, *J.C.S.*, 1945, 877; Peat, Bourne and S. A. Barker, *Nature*, 1948, **161**, 127.

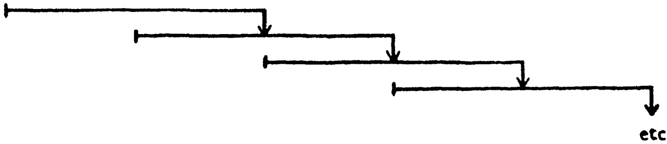
Glycogen

The discrepancy between the molecular weight of glycogen determined either by the hydrolysis of the methylated polysaccharide (page 118) or by the periodate oxidation method, which gives results in most cases corresponding to a chain of twelve glucose units, and the particle weight determined by measurement of osmotic pressure* which gives results of $1\text{-}2 \times 10^6$, is more marked even than in the case of amylopectin. There is no component corresponding to the amylose fraction of starches but for various reasons our knowledge of the fine structure of glycogen is not so deep as for amylopectin. A laminated structure similar to that proposed for amylopectin has been suggested for glycogen but no direct evidence is available at present to support it. On the basis of enzymic degradations Meyer and Fuld† hold that a multiple branched structure explains the results. β-Amylase degrades the glycogen of the edible mussel (*Mytilus edulis*) to give about 50% of maltose and a product of high molecular weight which has an average chain length of 5·5 glucose units, since the methylated dextrin gave 18% of tetramethyl glucose on hydrolysis. Meyer supposes, therefore, that the outer branches consist of 6-7 glucose units joined by 1,4-α-links and that after the attack of β-amylase only one or two glucose units are left on these chains, and these furnish the new end groups. The branching points are

Simplified Representation of Glycogen (Meyer)



A = Reducing Group



---

* Carter and Record, *J.C.S.*, 1939, 624; Oakley and F. G. Young, *Biochem. J.*, 1936, **30**, 868.

† *Helv. Chim. Acta*, 1941, **24**, 375.

supposed to be 1,6-links which halt the attack of the β-amylase. Very short chains of about three glucose units are to be found between the branches. Such a model is claimed to explain why glycogen cannot take on a parallel arrangement in a micelle, but the same is true of amylopectin and a laminated structure would explain the facts equally well.

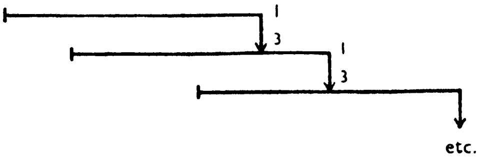
Enzymatic Synthesis of Glycogen

By the action of heart or liver phosphorylase on glucose 1-phosphate a polysaccharide like natural glycogen has been made* which gives a red colour with iodine and no X-ray pattern. It has been concluded that this synthesis is the result of the action of two enzymes, one, the ordinary phosphorylase synthesising the 1,4-links, and another supplementary enzyme in heart or liver, as yet not isolated in the pure state, responsible for 1,6-links,† an observation recalling the enzymic synthesis of amylopectin.

Xylan

The last polysaccharide to be dealt with in this Chapter, xylan, merits attention if only because it was the first polysaccharide for which a laminated structure was proposed.

Originally considered to be composed of a long chain of xylopyranose units joined through C_1 and C_4 by β-links as in cellulose, evidence was presented‡ that 2,3,5-trimethyl L-arabinose was produced on hydrolysis in such an amount as to give a calculated chain length of about 20 units of xylopyranose terminated by an L-arabofuranose residue. Removal of the arabinose by mild hydrolysis gave an arabinose-free xylan from which § trimethyl D-xylopyranose (7%) was isolated, again giving a chain length of about 18-19 units. The molecular weight of xylan was much higher than this figure would demand, osmotic pressure measurements indicating a value of upwards of 30,000, and a laminated structure was proposed. The point



---

* Bear and Cori, *J. Biol. Chem.*, 1941, **140**, 111.

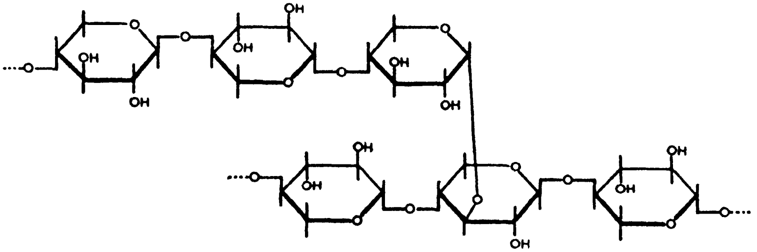
† Cori and Cori, *J. Biol. Chem.*, 1943, **151**, 57.

‡ Haworth, Hirst and Oliver, *J.C.S.*, 1934, 1917.

§ Bywater, Haworth, Hirst and Peat, *J.C.S.*, 1937, 1983.

of junction of one chain to its neighbour was found to be at the 3 position, for the monomethyl xylose which accompanied the trimethyl pentoses proved to be 2-methyl xylose.*

It should perhaps be stated that there is another way of interpreting the experimental results which led to the *postulation of* an arabofuranose end group in xylan.† If the xylan used *in the* original experiments happened to contain a small quantity of a branched chain araban, of the type so frequently found in woody tissues and associated with pectins,‡ such a mixture would be exceedingly difficult to separate. Since arabans ot the type in question on methylation and hydrolysis give one third of their hydrolysis products as trimethyl arabofuranose,§ comparatively little of the contaminating polysaccharide would be necessary. The mild hydrolysis carried out to prepare the arabinose-free xylan|| could have eliminated the branched chain araban, since it is readily hydrolysed, thus simulating the disappearance of an arabofuranose residue from one end of the xylan chain. Recently an araban-free esparto xylan¶ has been isolated and investigated, and the best representation of xylan at present is possibly by chains of about 40 D-xylopyranose units linked by β-links as shown, and attached to a neighbouring chain by a 1,3-linkage.



Cross Linking of Chains in Xylan

Some confirmation that a third covalency engages one of the xylopyranose units in the chain is given by treatment with periodic acid. Since periodic acid can attack the α-glycol residues of xylan**, one

---

* Bywater, Haworth, Hirst and Peat, *J.C.S.*, 1937, 1983.

† Hirst, *J.C.S.*, 1949, 522.

‡ Hirst, *J.C.S.*, 1942, 70.

§ Hirst and J. K. N. Jones, *J.C.S.*, 1947, 1221.

|| Haworth, Hirst and Oliver, *J.C.S.*, 1934, 1917.

¶ Chanda, Hirst and Percival (unpublished).

Jayme and Sätre, *Ber.*, 1942, **75, 1840; 1944, **77**, 242, 248.

would expect all the 1,4-linked xylopyranose residues, which have the adjacent free hydroxyl groups on C_2 and C_3, to be oxidised to the corresponding dialdehyde. Periodic acid could not attack the xylose residues linked at 1,4 and 3, because no α-glycol residues are present and the xylan oxidised with periodate should, therefore, on hydrolysis give one xylose residue per chain. Actually the above German authors obtained some xylose but attributed its presence to under-oxidation with periodate. An investigation in the Manchester laboratories involving separation of the xylose by paper chromatography (see page 168) has shown that the amount of xylose produced after periodate oxidation and hydrolysis is consistent with the type of branched chain or laminated structure proposed.*

---

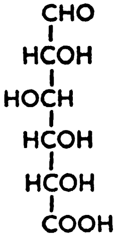
* Hirst, *J. Edin. Univ. Chem. Soc.*, 1946, 8, 34; *Nature*, 1947, **160**, 899.

CHAPTER VIII

THE URONIC ACIDS AND THE POLYURONIDES

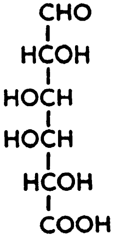
Simple Uronic Acids

An uronic acid is derived from a sugar by the oxidation of the primary alcoholic group to carboxyl. The chief uronic acids are D-glucuronic, D-galacturonic and D-mannuronic acids.



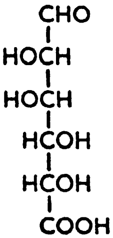
$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{HOCH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{COOH} \end{array}$$

D-Glucuronic acid



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{HOCH} \\ | \\ \mathrm{HOCH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{COOH} \end{array}$$

D-Galacturonic acid

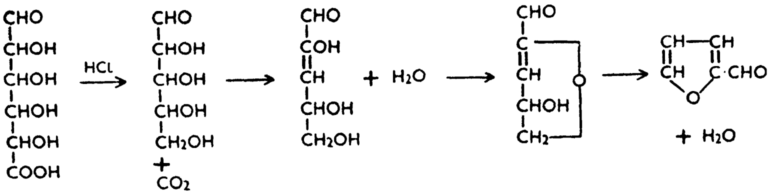


$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HOCH} \\ | \\ \mathrm{HOCH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{COOH} \end{array}$$

D-Mannuronic acid

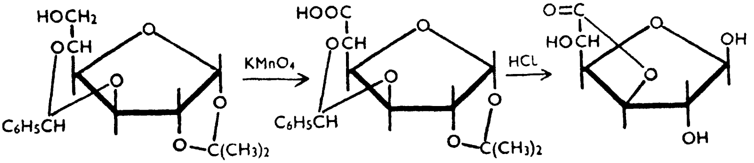
Although not as a rule found in the free state, these acids are very widely distributed in the plant and animal kingdoms. Thus glucuronic acid is found combined in many plant gums, such as gum arabic, damson gum and egg plum gum, in the blood anticoagulant heparin, in chondroitin sulphuric acid, and in many of the specific polysaccharides of bacteria. Galacturonic acid is the building unit of pectic acid, the main constituent of pectin, and is found in plant mucilages and in bacterial polysaccharides, while mannuronic acid is the building unit of the important seaweed polysaccharide alginic acid.

In many of their general properties the uronic acids resemble the parent sugars. They are reducing, can exist in α- and β-forms and may be converted into glycosides in the usual way. The carboxyl group can be esterified, it forms salts with inorganic and with organic bases, and some alkaloidal salts such as the cinchonine and brucine salts have been used for identification purposes. In the presence of strong mineral acids, the uronic acids give specific colour reactions with phenolic compounds like orcin (3,5-dihydroxy toluene) and naphthoresorcin. When uronic acids are boiled with hydrochloric acid (19%) carbon dioxide is evolved and furfural is produced; the evolution of carbon dioxide is quantitative and is used as a means of estimation.*



D-Glucuronic Acid

By the reduction of D-saccharolactone with sodium amalgam (page 19) Fischer prepared glucuronic acid. Among other methods which have been devised, the oxidation of 1,2-monoacetone 3,5-benzylidene glucose† (page 58) and of 1,2,3,4-tetraacetyl glucose with permanganate‡ may be mentioned. From the product of the first oxidation, mild acid hydrolysis removes the substituent groupings and treatment of the second with barium hydroxide yields barium D-glucuronate.



1,2-Monoacetone 3,5-benzylidene glucose

D-Glucurone (β-form)

Glucuronic acid forms the crystalline lactone, glucurone, so readily that the free acid is very difficult to isolate in the pure state. It has been shown by methylation studies that glucurone contains two five membered rings,§ a fact which recalls the stability of the 3,6-anhydro methylglucofuranosides (page 68).

Glucuronic acid is produced in nature as a result of one of the detoxication mechanisms which operate in mammals when excessive amounts of poisons such as phenolic substances, have been ingested in the diet. When a dog is fed with borneol, or camphor, the body

---

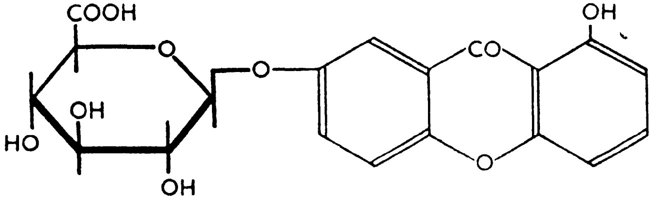
[*] Tollens and Lefèvre, *Ber.*, 1907, **40**, 4517; McCready, Swenson and Maclay, *Ind. Eng. Chem. Anal.*, 1946, 290.

[†] Zervas and Sessler, *Ber.*, 1933, **66**, 1326.

[‡] Stacey, *J.C.S.*, 1939, 1529.

[§] Smith, *J.C.S.*, 1944, 544.

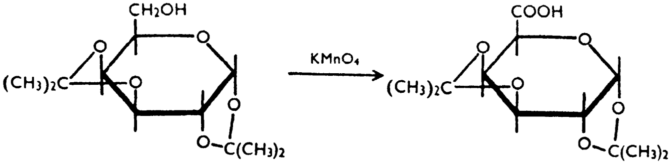
gets rid of the toxic substance by its excretion as borneol glucuronide, the camphor being reduced first to borneol. Cows fed on mango leaves in India excrete quantities of the magnesium salt of euxanthic acid, which is still used in the United Provinces as the pigment, Indian Yellow or " Piuiri ". It should be mentioned perhaps that other detoxication processes proceed at the same time, such as the conversion of phenols to ethereal sulphates and the removal of benzoic acid in combination with glycine as hippuric acid. Nevertheless detoxication using glucuronic acid is highly important. The poisons are converted into acidic substances in this way because the kidneys are able to function rapidly in dealing with acids.



Euxanthic acid

D-Galacturonic Acid

The uronic acid derived from D-galactose may be obtained by oxidising with permanganate either diacetone galactose (page 55) or 1,2,3,4-tetraacetyl galactose, followed by the appropriate hydrolyses.

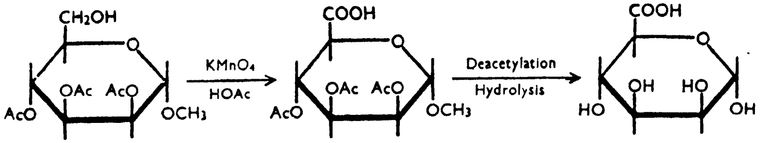


Diacetone galacturonic acid

By contrast with glucuronic acid it is difficult to convert the acid into the corresponding lactone; inspection of a model shows that, as for the 3,6-anhydro galactose derivatives (page 68), unlike the case of glucurone, two furanoid rings cannot be accommodated. Even if the galacturonic acid be assumed to have the pyranose structure, by analogy with the 3,6-anhydro methylhexosides, a derivative containing a five and a six membered ring would not be particularly stable.

D-Mannuronic Acid

The reduction of mannosaccharodilactone gives mannuronic acid and other methods of preparation are the oxidation either of 2,3-monoacetone α-methylmannoside* or of 2,3,4-triacetyl α-methylmannoside† (I) followed by hydrolysis.

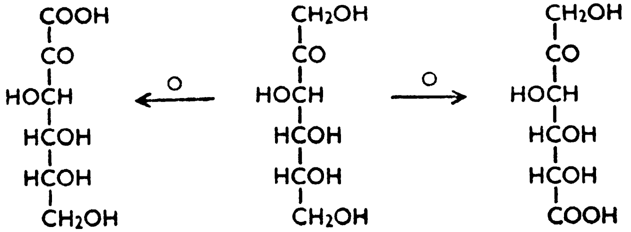


I

α-D-Mannuronic acid

Ketouronic Acids

Two ketouronic acids can be derived from each ketohexose, so that both 2-keto-D-gluconic acid and 5-keto-L-gulonic acid (synonymous with 5-keto-L-mannonic acid) could arise from fructose. 2-Keto-D-gluconic acid is usually referred to as fructuronic acid but this term is obviously misleading. It may be prepared from glucosone (page 8) by oxidation with bromine, and also by the oxidation of β-diacetone fructose (page 56). When gluconic acid is acted upon in neutral solution by *Bact. gluconicum*, 2-keto-gluconic acid is obtained; this acid has also been reported as a constituent of the polysaccharide of the red seaweed *Chondrus crispus*‡ and an isomer of 5-keto-gulonic acid, namely 2-ketogulonic acid, is an intermediate in the preparation of ascorbic acid (page 204).



2-Ketogluconic acid Fructose 5-Keto-L-gulonic acid

Aldobiuronic Acids

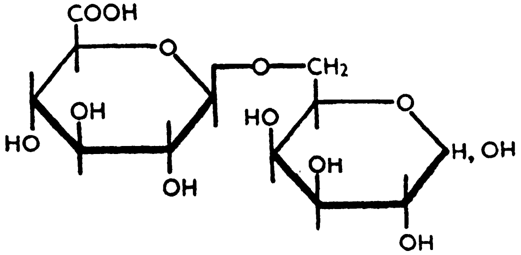
It frequently happens that when plant polysaccharides, such as gum arabic, are hydrolysed with acids, a more resistant portion is isolated in the form of a disaccharide, one half of which is a uronic acid. Compounds of this type form important clues to the structure of many natural polysaccharides. Thus from gum arabic and egg plum gum an aldobiuronic acid has been isolated, which has been proved by methylation studies and by synthesis* to be 6-[β-D-glucuronosido]-D-galactose (I).

---

* Ault, Haworth and Hirst, *J.C.S.*, 1935, 517.

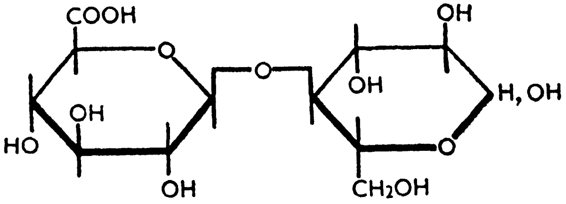
† Stacey, *J.C.S.*, 1939, 1529.

‡ E. G. Young and Rice, *J. Biol. Chem.*, 1946, **164**, 35.



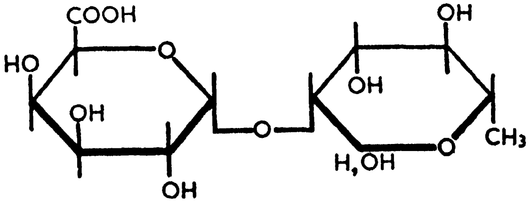
(I)

From the Type III pneumococcus specific polysaccharide, cellobiuronic acid, 4-[β-D-glucuronosido]-D-glucose (II) has been isolated.†



(II)

Slippery Elm mucilage yields 2-[α-D-galacturonosido]-L-rhamnose,‡ (III),



(III)

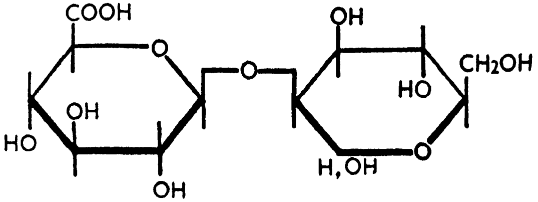
---

* Hotchkiss and Goebel, *J. Amer. Chem. Soc.*, 1936, **58**, 858.

† Heidelberger and Goebel, *J. Biol. Chem.*, 1926, **70**, 613; 1927, **74**, 613.

‡ Gill, Hirst and J. K. N. Jones, *J.C.S.*, 1939, 1469.

and damson gum* 2[β-D-glucuronosido]-D-mannose (IV).



(IV)

Polyuronides

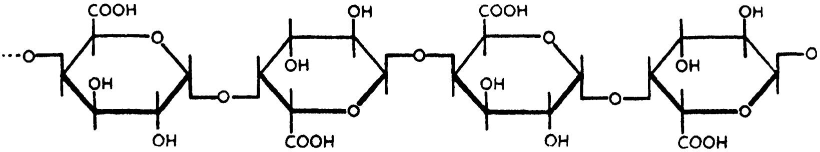
Taken literally, the term polyuronide, means a polysaccharide constructed entirely of uronic acid residues. Such a definition is too narrow, however, and any homogeneous polysaccharide containing uronic acid units among its building stones may be classed as a polyuronide. Since a great many compounds come under this wider definition it will be possible to consider a few examples only.

Two important polysaccharides which appear to be made up entirely of uronic acid units are pectic acid and alginic acid.

Pectic Acid

The main component of the substance called pectin, which occurs in nearly all land plant materials, particularly in fruit and young tissues and also in wood, is an acidic substance called pectic acid. Associated with the pectic acid, which, in the natural state, is partially esterified with methanol, are to be found a galactan (page 213) and an araban.

The essential feature of pectic acid is a chain of α-D-galacturonic acid units of pyranose form, linked through the 1,4-positions, a structure suggested by Link†, by Schneider‡ and substantiated by the work of Beavan and J. K. N. Jones§ and of Luckett and Smith.||



Chain of α-D-Galacturonic acid units in Pectic acid

---

* Hirst and J. K. N. Jones, *J.C.S.*, 1938, 1174.

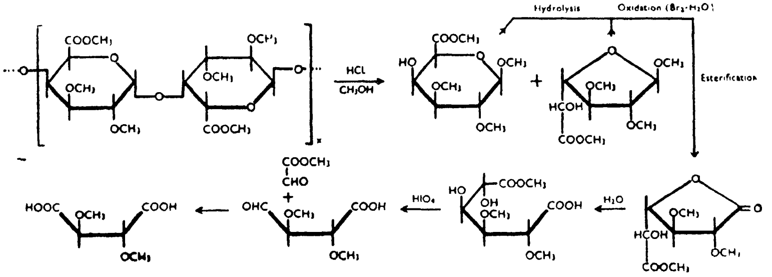
† *J. Biol. Chem.*, 1935, **109**, 293.

‡ *Ber.*, 1937, **70**, 1617.

§ *Chem.* and *Ind.*, 1939, **58**, 363; *J.C.S.*, 1947, 1218.

|| *J.C.S.*, 1940, 1106; 1114, 1509.

The methylation of degraded pectic acids, from apple, strawberry, and other pectins, by the thallium method (page 51) and hydrolysis with methanolic hydrogen chloride, gave a mixture of the pyranose and furanose forms of 2,3-dimethyl methylgalacturonoside methyl ester and a similar result was found for the methylated polygalacturonic acid of citrus pectin.* That the methyl groups were present on C_2 and C_3 was shown by oxidation to the L(+)-dimethoxysuccinic acid.

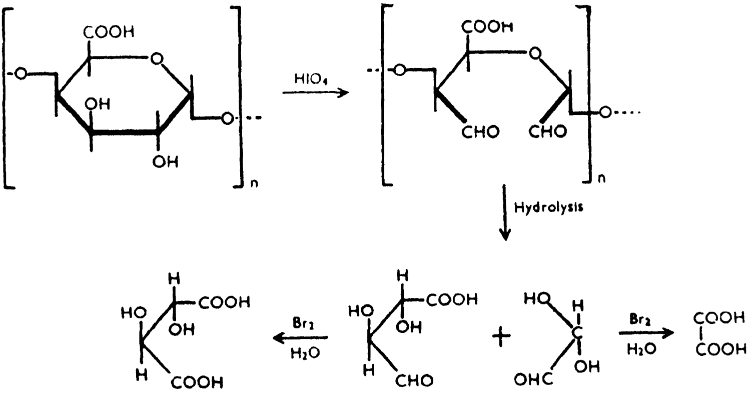


L(+)-Dimethoxysuccinic acid

γ-Lactone methyl ester of 2,3 dimethyl mucic acid

Oxidation of pectic acid with periodic acid had been used previously† to give D(–)-tartaric acid as shown in the accompanying scheme.

Oxidation of Pectic acid with Periodic acid



D(–)-Tartaric acid

---

* *J.C.S.*, 1940, 1106; 1114, 1509.

† Levene and Kreider, *J. Biol. Chem.*, 1937, **120**, 591.

This result is in agreement with those already quoted and shows that the hydroxyl groups on C_4 and C_5 were not available for attack by the periodic acid since D(−)-tartaric acid could not have come from any other source. The alternative formulations of pyranose units linked 1,4 or furanose units linked 1,5 are not distinguished by these methods, but the stability of pectic acid to hydrolysis strongly suggests the former possibility and this is supported by the results of X-ray analysis. The very high specific rotation of sodium pectate, $[\alpha]_D$*ca* +280°, points to the presence of α-linkages.

Measurements of the molecular weight of pectic acid gave values of over 200,000 for nitrated apple and citrus pectins, and Schneider* by means of osmotic pressure and viscosity measurements puts the value at between 35,000-100,000, and measurements with the ultracentrifuge gave results of the same order.† Apple, pear and plum pectins gave values from 25,000-30,000 and for orange pectin 40,000-50,000.

In the investigations of methylated citrus pectin and of degraded pectic acid no " end group " of 2,3,4-trimethyl galacturonic acid was found although it would perhaps be premature to suggest that the D-galacturonic acid units were arranged in closed loops.

As indicated earlier, an araban and a galactan are associated with pectic acid in the substance known as pectin. Loss of carbon dioxide from D-galacturonic acid would give L-arabinose and it is necessary to consider whether the araban might be a decarboxylation product of pectic acid. The researches of Hirst and J. K. N. Jones‡ show that in the araban the arabinose units are furanose in character and that the compound has a highly branched structure and prove that the direct decarboxylation theory is untenable. Similarly for the galactan (page 213) associated with pectin; although in it the galactopyranose units are linked 1,4, the links are β and not α as in pectic acid, so that pectic acid could not be formed in the plant by oxidation of the galactan.

Alginic Acid

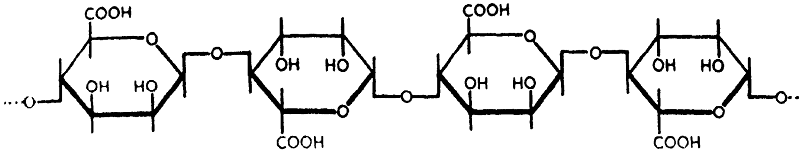
In the brown seaweeds (*Phaeophyceæ*) the main structural carbohydrate is not cellulose as in land plants but alginic acid, a substance which is proving to be of some commercial importance. This polymannuronide is readily extracted with sodium carbonate solution and is obtainable either as an insoluble calcium salt on precipitation with calcium chloride or as the insoluble acid on acidification. D-Mannuronic acid is the only residue so far known to be present and is joined precisely as is glucose in cellulose by 1,4-β-links. Owing to the extreme stability of alginic acid, structural studies have been conducted on a degraded alginic acid which has been methylated and hydrolysed to give 2,3-dimethyl mannuronic acid.* The structure of this substance was indicated by oxidation with bromine to a dimethyl mannosaccharic acid, followed by oxidation with periodic acid and further oxidation of the semialdehyde of the dimethoxysuccinic acid so obtained. This gave *meso*dimethoxysuccinic acid from which it is clear that the methoxyl groups must have been on adjoining carbon atoms, namely either C_2 and C_3, or C_4 and C_5. Since the methyl ester of the dimethyl methylmannuronoside underwent a change in specific rotation on hydrolysis it was evident that an oxide ring was present so that substitution on C_4 and C_5 is excluded. It is concluded, therefore, that in alginic acid the two free hydroxyl groups are on C_2 and C_3. The very marked stability to hydrolysis of the polyuronide is a clear indication that the rings are pyranose, hence the proposed structure. The authors have pointed out that since this work was carried out on a degraded alginic acid, the possibility of the existence of branched chains in the original polyuronide cannot be dismissed, although the results of X-ray studies would seem to discount any high degree of branching.

---

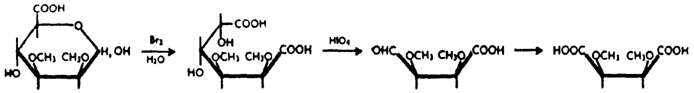
* *Ber.*, 1936, **69**, 2530.

† Svedberg and Gralén, *Nature*, 1938, **142**, 261.

‡ *J.C.S.*, 1938, 496; 1947, 1221.



Chains of β-D-Mannuronic acid units in Alginic acid



2,3-Dimethyl
mannuronic acid

i-Dimethoxysuccinic
acid

The formal resemblance between alginic acid and cellulose is obvious but W. T. Astbury† has shown that the periodicity along the

---

* Hirst, J. K. N. Jones and W. O. Jones, *J.C.S.*, 1939, 1880.

† *Nature*, 1945, **155**, 667.

This result is in agreement with those already quoted and shows that the hydroxyl groups on C_4 and C_5 were not available for attack by the periodic acid since D(–)-tartaric acid could not have come from any other source. The alternative formulations of pyranose units linked 1,4 or furanose units linked 1,5 are not distinguished by these methods, but the stability of pectic acid to hydrolysis strongly suggests the former possibility and this is supported by the results of X-ray analysis. The very high specific rotation of sodium pectate, $[\alpha]_D$ *ca* +280°, points to the presence of α-linkages.

Measurements of the molecular weight of pectic acid gave values of over 200,000 for nitrated apple and citrus pectins, and Schneider* by means of osmotic pressure and viscosity measurements puts the value at between 35,000-100,000, and measurements with the ultracentrifuge gave results of the same order.† Apple, pear and plum pectins gave values from 25,000-30,000 and for orange pectin 40,000-50,000.

In the investigations of methylated citrus pectin and of degraded pectic acid no " end group " of 2,3,4-trimethyl galacturonic acid was found although it would perhaps be premature to suggest that the D-galacturonic acid units were arranged in closed loops.

As indicated earlier, an araban and a galactan are associated with pectic acid in the substance known as pectin. Loss of carbon dioxide from D-galacturonic acid would give L-arabinose and it is necessary to consider whether the araban might be a decarboxylation product of pectic acid. The researches of Hirst and J. K. N. Jones‡ show that in the araban the arabinose units are furanose in character and that the compound has a highly branched structure and prove that the direct decarboxylation theory is untenable. Similarly for the galactan (page 213) associated with pectin; although in it the galactopyranose units are linked 1,4, the links are β and not α as in pectic acid, so that pectic acid could not be formed in the plant by oxidation of the galactan.

Alginic Acid

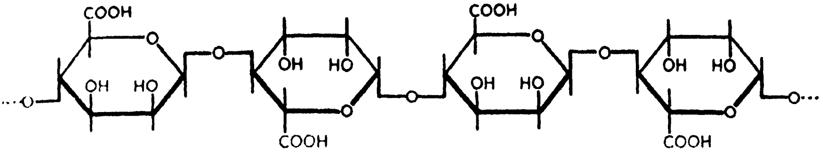
In the brown seaweeds (*Phaeophyceæ*) the main structural carbohydrate is not cellulose as in land plants but alginic acid, a substance which is proving to be of some commercial importance. This polymannuronide is readily extracted with sodium carbonate solution

* *Ber.*, 1936, **69**, 2530.

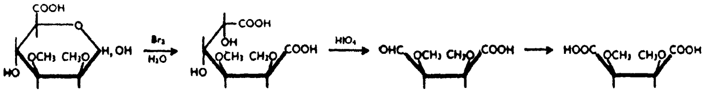
† Svedberg and Gralén, *Nature*, 1938, **142**, 261.

‡ *J.C.S.*, 1938, 496; 1947, 1221.

and is obtainable either as an insoluble calcium salt on precipitation with calcium chloride or as the insoluble acid on acidification. D-Mannuronic acid is the only residue so far known to be present and is joined precisely as is glucose in cellulose by 1,4-β-links. Owing to the extreme stability of alginic acid, structural studies have been conducted on a degraded alginic acid which has been methylated and hydrolysed to give 2,3-dimethyl mannuronic acid.* The structure of this substance was indicated by oxidation with bromine to a dimethyl mannosaccharic acid, followed by oxidation with periodic acid and further oxidation of the semialdehyde of the dimethoxysuccinic acid so obtained. This gave *meso*dimethoxysuccinic acid from which it is clear that the methoxyl groups must have been on adjoining carbon atoms, namely either C_2 and C_3, or C_4 and C_5. Since the methyl ester of the dimethyl methylmannuronoside underwent a change in specific rotation on hydrolysis it was evident that an oxide ring was present so that substitution on C_4 and C_5 is excluded. It is concluded, therefore, that in alginic acid the two free hydroxyl groups are on C_2 and C_3. The very marked stability to hydrolysis of the polyuronide is a clear indication that the rings are pyranose, hence the proposed structure. The authors have pointed out that since this work was carried out on a degraded alginic acid, the possibility of the existence of branched chains in the original polyuronide cannot be dismissed, although the results of X-ray studies would seem to discount any high degree of branching.



Chains of β-D-Mannuronic acid units in Alginic acid



2,3-Dimethyl mannuronic acid

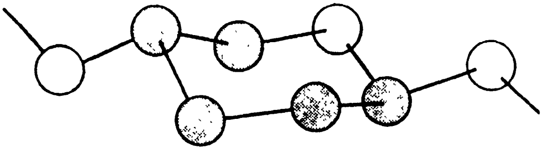
i-Dimethoxysuccinic acid

The formal resemblance between alginic acid and cellulose is obvious but W. T. Astbury† has shown that the periodicity along the fibre axis disclosed by X-ray studies is only 8·7Å instead of the value of 10·3Å characteristic of cellulose (page 109). This is explained by the assumption that the glycosidic oxygen residues are joined at different angles to the pyranose rings according as to which strainless Sachse " chair " form configuration is adopted. The consequence of this is that instead of the two rings making an angle of about 20° with one another as in cellulose, in the alternative structure which appears to hold for alginic acid the pyranose rings are inclined at an angle of approximately 90°. This leads to a greater irregularity of structure and a buckling effect, although it must be remembered that alginic acid, because of the 1,4-β-linkages, is essentially a " straight chain " polysaccharide like cellulose. X-ray studies on pectic acid and sodium pectate* indicate that the same phenomenon occurs there also.

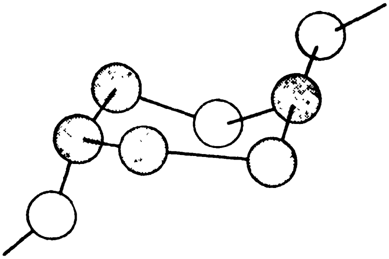
---

* Hirst, J. K. N. Jones and W. O. Jones, *J.C.S.*, 1939, 1880.

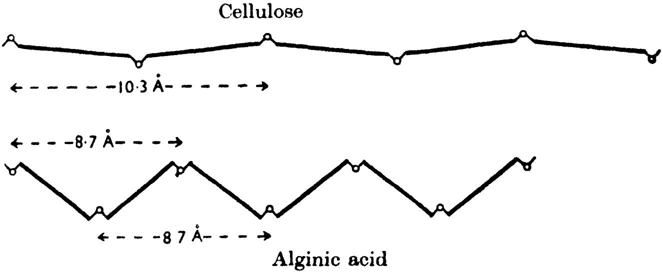
† *Nature*, 1945, **155**, 667.



In Cellulose



In Alginic acid



Molecular weight determinations show that alginic acid is a large molecule, a value of 185,000 having been reported for sodium alginate determined by the osmotic pressure method.

---

* Palmer and Hartzog, *J. Amer. Chem. Soc.*, **1945**, **67**, 2122.

For a natural polysaccharide alginic acid has certain unique properties which should be mentioned briefly. Although insoluble in water, alginic acid is readily soluble in sodium hydroxide or sodium carbonate to give viscous solutions of sodium alginate. When solutions of the sodium salt are treated with salts of polyvalent metals, such as calcium chloride, an insoluble calcium alginate is precipitated, the calcium atoms holding a number of chains together by ionic cross linkages. Viscous sodium alginate solution can therefore be spun into fibres of calcium alginate. Calcium alginate fibres as such are not particularly useful for the manufacture of textile materials, since alkalis such as sodium carbonate will dissolve them, but because they are non-inflammable, calcium alginate fabrics may find uses as furnishings in cinemas and similar places where high fire risks are present. By incorporating beryllium salts J. B. Speakman has obtained alginate fibres which, owing to the co-ordinating power of the beryllium atoms, withstand the action of sodium carbonate and soap solutions. The disappearing thread technique introduced by Speakman* holds out considerable commercial prospects. By weaving calcium alginate together with wool and scouring out the calcium alginate fibres with soda, very light-weight woollen fabrics can be made: twistless cotton can be woven by the use of calcium alginate as a support to overcome the natural twist and dissolving out the alginate rayon afterwards, and a great variety of fancy designs can be introduced into rayon and silk textiles; there have also been important developments in hosiery manufacture. Sodium alginate, marketed as " Manucol " finds applications as an emulsifier and thickener in the food and cosmetics industries; and since dried seaweed contains on an average something like 30% of alginic acid many industrial applications await a solution of the problems of seaweed harvesting.

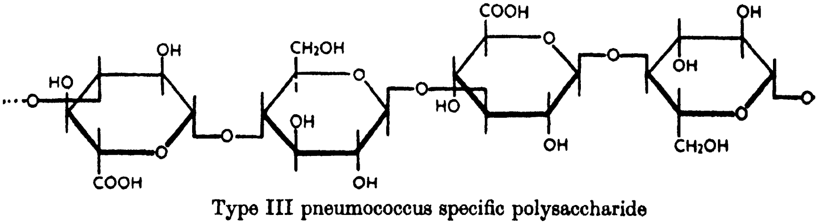
Bacterial Polyuronides

Many bacteria are surrounded by a capsule of a polysaccharide nature, and particular polysaccharides isolated from dried bacteria have been found to give specific agglutination or precipitation reactions with the antisera obtained by the action of the bacteria or bacterial strain concerned on animals. The conferring of immunity on an animal by the antiserum, which contains substances called antibodies, protein in nature and belonging to the globulin class, is evidently brought about by an agglutination process, which renders the bacteria responsible for the infection susceptible to destruction by the natural protective mechanisms of the host. The production of antibodies can be brought about by the stimulus of antigens, and a small amount of an antigen can, when present in the body, produce large quantities of the appropriate antibody. The specific polysaccharides referred to are not themselves antigens but, since in combination with protein they become antigenic, they are called haptens. When the appropriate antigenic polysaccharide-protein complex is injected into the blood stream of an animal, antibodies are produced in response, which confer immunity against the particular bacterial infection concerned. The study of the polysaccharides of this group is therefore of great importance in the difficult field of immunology, where much work remains to be done.*

---

* *Nature*, 1945, **155**, 655.

The specific polysaccharide of pneumococcus Type III contains glucose and glucuronic acid in the ratio of approximately 1 :1, and from it cellobiuronic acid (page 155) is produced on hydrolysis. The polysaccharide was shown to be composed of cellobiuronic acid units linked through 1,3-positions by β-links.† This structure was elucidated by subjecting the methylated polyuronide to catalytic hydrogenation, this process converting the carboxylic ester groups into primary alcoholic residues. Hydrolysis gave 2,3,6-trimethyl glucose and 2,4-dimethyl glucose (from the glucuronic acid portion) in equal amounts, indicating the point of union to be C_3. The polyuronide being laevorotatory and becoming dextrorotatory under conditions which split the 1,3-link but not the 1,4-glucuronosidic bond, pointed to the assignment of the β-configuration as shown in the formula.



Type III pneumococcus specific polysaccharide

The specific polysaccharide of the Pneumococcus Type VIII is related to that just discussed, in that the aldobiuronic acid is also cellobiuronic acid, but the ratio of glucose to glucuronic acid is higher, namely 3 : 1.

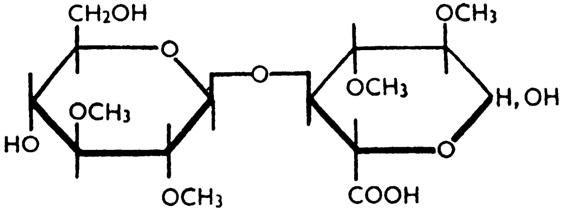
---

* See Stacey, " Aspects of Immunochemistry ", *Quart. Rev. Chem. Soc.*, 1947, **2**, 179; **3**, 1.

† Reeves and Goebel, *J. Biol. Chem.*, 1941, **139**, 511.

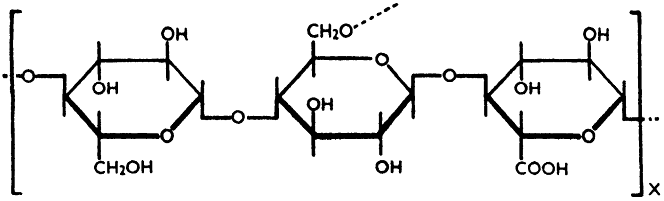
By the oxidation of cellulose with dinitrogen tetroxide, polyuronides have been obtained,* varying numbers of the primary alcoholic groups being oxidised to carboxyl groups depending on the conditions. Complete oxidation yields a substance of fibrous structure resembling alginic acid in that it forms a soluble sodium salt which is precipitated due to cross-linking of chains by the addition of barium chloride. Some of the partially carboxylated celluloses were found† to precipitate pneumococcus antisera (Types III and VIII).

The capsular polyuronide of *Rhizobium radicicolum*, a nitrogen fixing organism of leguminous plants such as clover, which was found to give reactions with pneumococcus antisera also gives rise to glucose and glucuronic acid on hydrolysis. On hydrolysing the methylated polyuronide a tetramethyl cellobiuronic acid was isolated‡ to which the following formula was assigned, since complete



Tetramethyl cellobiuronic acid

methylation and hydrolysis gave 2,3,4,6-tetramethyl glucose and 2,3-dimethyl glucuronic acid, whilst simple hydrolysis gave the latter substance and 2,3-dimethyl glucose. Since the methylated polyuronide gave on hydrolysis equal parts of 2,3,6-trimethyl glucose, 2,3-dimethyl glucose and 2,3-dimethyl glucuronic acid and the linkages from rotational evidence appear to be β, the repeating unit of the polysaccharide is envisaged as shown.



Repeating unit of *Rhizobium* polysaccharide

---

* Yackel and Kenyon, *J. Amer. Chem. Soc.*, 1942, **64**, 121.

† Heidelberger and Hobby, *Proc. Nat. Acad. Sci.*, 1942, **28**, 516.

‡ Schlüchterer and Stacey, *J.C.S.*, 1945, 776.

Plant Gums and Mucilages

The huge number of plant polysaccharides such as the hemicelluloses, plant gums and mucilages which contain uronic acid residues and must therefore be classed as polyuronides, makes it impossible to consider many examples. Owing to the complexities of structure and the difficulty of separating homogeneous substances, most of the research on substances of this type is in the development stage and detailed discussion would be inappropriate here. The so-called hemicelluloses which occur along with cellulose in plant tissues contain variable quantities of a uronic acid, usually glucuronic acid or a methoxyglucuronic acid, often associated with xylose units, although other sugars such as glucose, mannose and L-arabinose are also found.*

In mucilaginous seed polysaccharides such as those of the plantain and linseed families the uronic acid present is D-galacturonic acid and the same is true of the slippery elm mucilage made from the bark of the tree *Ulmus fulva*. From this mucilage on hydrolysis, equal parts of D-galacturonic acid, L-rhamnose, and D-galactose are obtained,† and the aldobionic acid produced on partial hydrolysis is 2-[α-D-galacturonosido]-L-rhamnose identical with the aldobiuronic acid of flax seed mucilage. When methylated slippery elm mucilage was hydrolysed,‡ 2,3-dimethyl D-galacturonic acid (4 parts), tetramethyl galactopyranose (2 parts), 2,4,6-trimethyl galactose (1 part), 2,3,6-trimethyl galactose (1 part), 3,4-dimethyl rhamnose (2 parts), 4-methyl rhamnose (2 parts), and a trace of 2,3,4-trimethyl galacturonic acid were obtained. This result illustrates the complexity of this mucilage which clearly contains a number of branched chains. It will be observed that there are 1,2-, 1,3- and 1,4-linkages in the same molecule and that the galacturonic acid units are apparently joined through C_1 and C_4 as in pectic acid.

The plant gums are also very complex polyuronides as the researches of Smith on gum arabic have shown. The aldobiuronic acid isolated on the appropriate acid hydrolysis of gum arabic is known to be 6-[β-D-glucuronosido]-D-galactose. Gum arabic is a salt which on acidification is converted into the corresponding acid, arabic acid, and this on boiling with water suffers autohydrolysis.

---

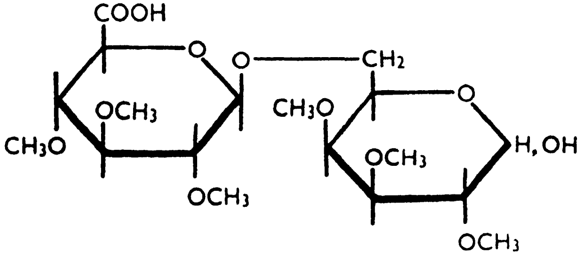
* See Anderson and Sands, *Advances in Carbohydrate Chemistry,* 1945, 1, 329.

† Gill, Hirst and J. K. N. Jones, *J.C.S.*, 1939, 1409.

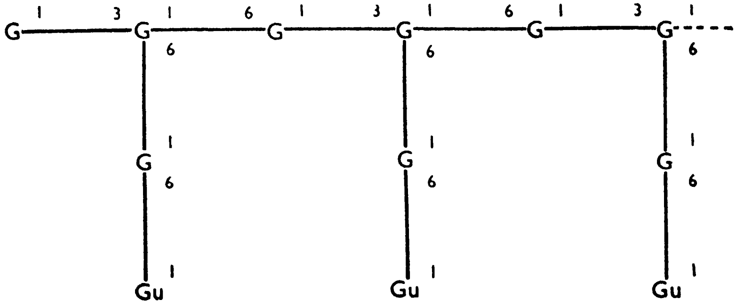
‡ Gill, Hirst and J. K. N. Jones, *J.C.S.*, 1946, 1025.

During this process L-arabinose, L-rhamnose and 3-[galactosido]-L-arabinose together with a degraded arabic acid were isolated.*

The hydrolysis of methylated degraded arabic acid gave tetramethyl galactopyranose (1 part), 2,3,4-trimethyl galactose (5 parts), 2,4-dimethyl galactose (3 parts) and 2,3,4-trimethyl glucuronic acid (3 parts). Prolonged autohydrolysis of degraded arabic acid gave 3-[D-galactosido]-D-galactose indicating the presence of two contiguous galactose units joined by the 1,3-link. The degraded gum therefore has a minimum of nine galactopyranose residues, one of which is an end group, and three end groups of glucopyruronic acid. Both the hydroxyl groups on C_6 and C_3 in the galactose units are involved in linkages between the galactose residues. By the cautious hydrolysis of methylated degraded arabic acid a hexamethyl aldobiuronic acid was obtained. This was proved to be hexamethyl 6-[β-D-glucuronosido]-D-galactose, thus showing that a galactose unit joined by a 1,6-link must be interposed between the glucuronic acid end group and the main chain. It is thus possible to write a structure for the degraded arabic acid, or rather for the repeating



Hexamethyl 6-[β-D-glucuronosido]-D-galactose



One possible diagrammatic representation of degraded Arabic acid
G = D-Galactopyranose Gu = D-Glucopyruronic acid

---

* Smith, *J.C.S.*, 1939, 744.

Plant Gums and Mucilages

The huge number of plant polysaccharides such as the hemicelluloses, plant gums and mucilages which contain uronic acid residues and must therefore be classed as polyuronides, makes it impossible to consider many examples. Owing to the complexities of structure and the difficulty of separating homogeneous substances, most of the research on substances of this type is in the development stage and detailed discussion would be inappropriate here. The so-called hemicelluloses which occur along with cellulose in plant tissues contain variable quantities of a uronic acid, usually glucuronic acid or a methoxyglucuronic acid, often associated with xylose units, although other sugars such as glucose, mannose and L-arabinose are also found.*

In mucilaginous seed polysaccharides such as those of the plantain and linseed families the uronic acid present is D-galacturonic acid and the same is true of the slippery elm mucilage made from the bark of the tree *Ulmus fulva*. From this mucilage on hydrolysis, equal parts of D-galacturonic acid, L-rhamnose, and D-galactose are obtained,† and the aldobionic acid produced on partial hydrolysis is 2-[α-D-galacturonosido]-L-rhamnose identical with the aldobiuronic acid of flax seed mucilage. When methylated slippery elm mucilage was hydrolysed,‡ 2,3-dimethyl D-galacturonic acid (4 parts), tetramethyl galactopyranose (2 parts), 2,4,6-trimethyl galactose (1 part), 2,3,6-trimethyl galactose (1 part), 3,4-dimethyl rhamnose (2 parts), 4-methyl rhamnose (2 parts), and a trace of 2,3,4-trimethyl galacturonic acid were obtained. This result illustrates the complexity of this mucilage which clearly contains a number of branched chains. It will be observed that there are 1,2-, 1,3- and 1,4-linkages in the same molecule and that the galacturonic acid units are apparently joined through C_1 and C_4 as in pectic acid.

The plant gums are also very complex polyuronides as the researches of Smith on gum arabic have shown. The aldobiuronic acid isolated on the appropriate acid hydrolysis of gum arabic is known to be 6-[β-D-glucuronosido]-D-galactose. Gum arabic is a salt which on acidification is converted into the corresponding acid, arabic acid, and this on boiling with water suffers autohydrolysis.

---

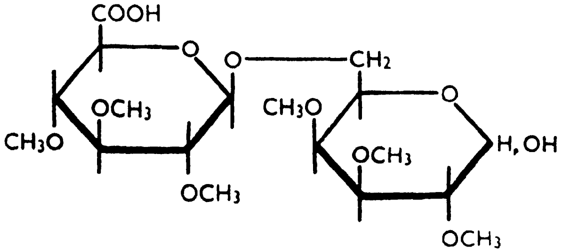
* See Anderson and Sands, *Advances in Carbohydrate Chemistry*, 1945, 1, 329.

† Gill, Hirst and J. K. N. Jones, *J.C.S.*, 1939, 1409.

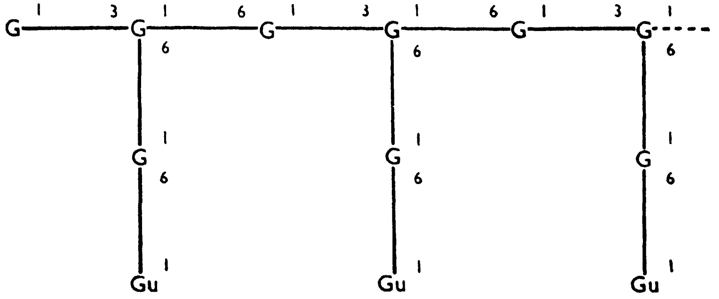
‡ Gill, Hirst and J. K. N. Jones, *J.C.S.*, 1946, 1025.

During this process L-arabinose, L-rhamnose and 3-[galactosido]-L-arabinose together with a degraded arabic acid were isolated.*

The hydrolysis of methylated degraded arabic acid gave tetramethyl galactopyranose (1 part), 2,3,4-trimethyl galactose (5 parts), 2,4-dimethyl galactose (3 parts) and 2,3,4-trimethyl glucuronic acid (3 parts). Prolonged autohydrolysis of degraded arabic acid gave 3-[D-galactosido]-D-galactose indicating the presence of two contiguous galactose units joined by the 1,3-link. The degraded gum therefore has a minimum of nine galactopyranose residues, one of which is an end group, and three end groups of glucopyruronic acid. Both the hydroxyl groups on C_6 and C_3 in the galactose units are involved in linkages between the galactose residues. By the cautious hydrolysis of methylated degraded arabic acid a hexamethyl aldobiuronic acid was obtained. This was proved to be hexamethyl 6-[β-D-glucuronosido]-D-galactose, thus showing that a galactose unit joined by a 1,6-link must be interposed between the glucuronic acid end group and the main chain. It is thus possible to write a structure for the degraded arabic acid, or rather for the repeating



Hexamethyl 6-[β-D-glucuronosido]-D-galactose



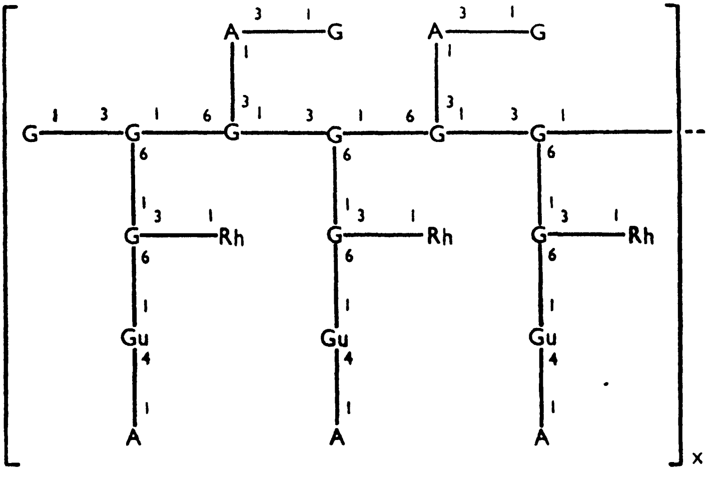
One possible diagrammatic representation of degraded Arabic acid
G = D-Galactopyranose Gu = D-Glucopyruronic acid

---

* Smith, *J.C.S.*, 1939, 744.

unit, since the molecular weight is not known. The structure given below is not the only possibility, however; instead of the main chain having alternate 1,3-1,6-linkages between the galactose residues, we could have all 1,6-links with 1,3-links for the side chains, or some compromise between the two extremes.

The hydrolysis of fully methylated arabic acid gave 2,3,4-trimethyl L-rhamnopyranose, 2,3,5-trimethyl L-arabofuranose and tetramethyl galactopyranose. These sugars must therefore appear as end groups, and it is these which are split off during autohydrolysis. In addition 2,5-dimethyl L-arabofuranose, 2,4-dimethyl galactopyranose, and 2,3,4-trimethyl- and 2,3-dimethyl-glucuronic acid were identified. No 2,3,4-trimethyl galactose appeared, so that the units which give rise to this product in the methylated degraded gum arabic must have links at C_3. The 2,5-dimethyl arabinose is related to the 3-[galactosido]-L-arabinose obtained on autohydrolysis, and the fact that a high proportion of 2,3-dimethyl glucuronic acid was present makes it evident that only a few of the glucuronic acid residues exist as end groups.* One formulation, constructed ignoring the small quantity of 2,3,4-trimethyl glucuronic acid isolated, is shown below, although there are other possibilities capable of fitting the facts.



A = L-arabofuranose
Rh = L-rhamnopyranose
One possible representation of the Repeating unit of Arabic acid

---

* Smith, *J.C.S.*, 1939, 1724; 1940, 1035; J. Jackson and Smith, *J.C.S.*, 1940, **74**, 79.

The studies of Hirst, J. K. N. Jones and their collaborators on other gums, such as damson, egg plum, cherry and almond tree gum, show the complexities of the group of compounds in question.*

As already pointed out the aldobiuronic acid of damson gum is 2-[D-glucuronosido]-D-mannose while egg plum and almond tree gum contain the same aldobiuronic acid as gum arabic. The reader may be forgiven if he regards the problem of the structure of the plant gums as one of extreme difficulty, but the progress with gum arabic and with other gums shows that the problems are not insuperable, and increased progress is certain to follow recent improvements in technique, in which chromatography, and especially the paper chromatogram, are outstanding examples.

The Separation of Mixtures of Sugars from Polysaccharide Hydrolysates by Chromatography and Related Methods

The technique of chromatography in recent years has been applied widely in organic chemistry to separate mixtures of such coloured compounds as the carotene pigments and chlorophylls. Attempts were made with some success to extend its application to mixtures of sugars by preparing coloured compounds such as the phenyl azobenzoyl esters. Such a method depends on the production of coloured bands on a column of alumina, silica gel or other absorbent, at different points on the column after developing with a suitable solvent. There are obvious disadvantages in such a method since the coloured esters have to be prepared, and after separation, decomposed again, resulting in loss of material.

A modification depends on the detection of the bands by extruding the column and brushing it with an indicator such as alkaline permanganate. Various substances have been used to prepare the columns in addition to silica and alumina, including hydrated magnesium silicates, calcium silicates, Florida clay and " Celite " (a diatomaceous earth). Sometimes it is necessary to carry out " blind " chromatography. The column is developed with known volumes of a chosen solvent, for example a mixture of chloroform and light petroleum if one is attempting to separate a mixture of methylated sugars, and the washing liquid is evaporated and the fractions examined, for example, by determining the refractive indices. The synthetic cellobiose octaacetate and gentiobiose octaacetate prepared by the sodium method (page 95) were separated in this way.

* *J. Soc. Dyers and Colourists*, 1947, **63**, 249; *J.C.S.*, 1942, 70; 1947, 1064, 1065; 1948, 120.

Very delicate separations have been made in the end group analyses of D. J. Bell[*] using a partition method between chloroform and water held in a silica gel column, in which it was found possible to separate 0·05 g. of tetramethyl glucose from 10 g. of trimethyl glucose.

J. K. N. Jones[†] achieved a separation of tetramethyl methylglucosides from the corresponding trimethyl derivatives with an alumina column; he was also able to separate partially trimethyl methylarabofuranosides from trimethyl methylxylopyranosides.

As a third illustration a method making use of the property that methylated sugars fluoresce in ultraviolet light[‡] may be mentioned. The mixture of methylated sugars is dissolved in benzene and the solution passed through a column of alumina. On developing with benzene the progress of the bands may be followed in ultraviolet light since the alumina is non-fluorescent. The fully methylated sugars are of course adsorbed the least strongly, since no free hydroxyl groups are present, and this band passes down the column first.

The separation of amino acids from the hydrolysis of proteins has been carried out in a remarkable manner using filter paper strips in an atmosphere of a suitable solvent.[§] It has been found possible to extend this technique to sugars. S. M. Partridge,[‖] using filter paper strips dipping into a trough containing *n*-butanol saturated with water, and maintained at a uniform temperature in an atmosphere of butanol, showed that mixtures of several sugars could be separated on a micro scale and identified by the rate of travel along the paper chromatogram. A micro spot of a solution containing the mixture of sugars is placed on the paper; after a period of two or three days the paper is dried and sprayed with a solution of ammoniacal silver nitrate and the positions of the reducing sugars are indicated by well-defined zones on the paper strip. Flood, Hirst and J. K. N. Jones[¶] have shown that this method can be used to estimate with accuracy the amounts of the different sugars present in a mixture. The strip is cut and the sugars extracted by boiling water and estimated by micro reducing power determinations; since by running on the same strip a separate portion of the unknown mixture, cutting it off and spraying with ammoniacal silver nitrate, the position of the spots on the portion to be used for quantitative analysis can be found. Mixtures of methylated sugars may be separated in a similar way and estimated with alkaline iodine on the micro-scale.* Such mixtures can also be separated on a larger scale by the use of a column of powdered cellulose and an automatic device for collecting the fractions.†

---

[*] *J.C.S.*, 1944, 473.

[†] *J.C.S.*, 1944, 333.

[‡] Norberg, Auerbach and Hixon, *J. Amer. Chem. Soc.*, 1945, **67**, 342.

[§] Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.

[‖] *Nature*, 1946, **158**, 270; *Biochem. J.*, 1948, **42**, 238.

[¶] *Nature*, 1947, **160**, 86; *J.C.S.*, 1948, 1679.

By methods such as this and a solvent extraction method by which fully methylated glycosides can be separated from the mixtures obtained on hydrolysing methylated polysaccharides with methanolic hydrogen chloride‡ many problems of structure can be studied with quantities of starting material that would have seemed fantastic ten years ago. It is likely therefore that the development of structural polysaccharide chemistry will be greatly accelerated by these new techniques.

---

* Hirst, Hough and J. K. N. Jones, *J.C.S.*, 1949, 928.

† Hirst, J. K. N. Jones and Wadman, *Nature*, 1948, **161**, 720; Hough, J. K. N. Jones and Wadman, *ibid.*, **162**, 448.

‡ Brown and J. K. N. Jones, *J.C.S.*, 1947, 1344.

CHAPTER IX

NATURAL GLYCOSIDES

NATURAL glycosides were known long before the preparation of the alkyl glycosides by Fischer; it is only necessary to recall the classical work of Liebig and Wöhler on the benzoyl radical employing oil of bitter almonds (1837) which was obtained by the enzymic hydrolysis of the crystalline glycoside amygdalin.

The sugar residue in most glycosides is D-glucose but other monosaccharides such as L-rhamnose, and, less frequently, D-fucose, D-galactose, and pentoses are present in some glycosides, as well as disaccharides like gentiobiose, primeverose and rutinose. The non-carbohydrate portion of the glycoside is generally called the aglucone or aglycone, thus methanol could be considered as the aglucone of the methylglucosides.

Accompanying the glycosides of plants there is an appropriate enzyme, or mixture of enzymes, capable of hydrolysing the glycoside. The enzyme is naturally not contained in the same cells as the glycoside, but is able to act either when the cells are bruised or at some particular stage in the life cycle of the plant. Almost without exception natural glycosides are members of the β-series. But there is an exception in floridoside, which is considered to be a glycerol α-D-galactoside* and is found in the red seaweed, *Rhodymenia palmata.* The evidence cited for its description as an α-galactoside includes the high optical rotation, failure to be hydrolysed by emulsin and its hydrolysis by bottom yeast. The galactose is attached to the secondary alcoholic group of the glycerol.

The enzyme named emulsin by Liebig and Wöhler which hydrolyses the amygdalin of bitter almonds is usually responsible for the hydrolysis of the plant glycosides; it is, however, not a pure β-glucosidase but according to the investigations of Helferich† can also hydrolyse β-D-galactosides, β-D-xylosides, α-D-mannosides, α-L-arabinosides and α-D-galactosides. Glycosides are hydrolysed by acids in the same way as the synthetic methylglucosides. Plant glycosides are found in the leaves and seeds of plants, and in the bark of trees, and they are exceedingly numerous. In the animal world also, we find such glycosides as cerebrosides in nerve and brain tissue and in the nucleoproteins nitrogen glycosides form part of the building units. Although these latter compounds will be considered briefly, the main purpose of this chapter is to review the most important types of plant glycosides. These compounds are usually crystalline solids, with a bitter taste and are frequently poisonous.

---

* Colin and Guègen, *Compt. rend.*, 1930, **191**, 163.

† Helferich and Winkler, *Z. physiol. Chem.*, 1932, **209**, 269; Helferich and Appel, *ibid.*, 1932, **205**, 231.

No single explanation is sufficient to account for the presence of glycosides in plants. It may be that harmful substances such as phenols are removed from certain sites of development during the growth of the plant by the agency of some kind of detoxication mechanism similar to that which takes place in animals when glucuronosides are formed (page 152). The extremely bitter taste of glycosides and, presumably, the poisonous nature of some of them, is in some cases a protection against attacks by animals. Possibly of greater importance is the fact that the plant is protected from bacterial invasion, if damage such as bruising takes place, by the presence of the glycoside and the enzyme appropriate for hydrolysis in neighbouring cells, since many glycosides release antiseptic substances such as phenols, or poisons such as prussic acid, on hydrolysis. It is significant that the bark of trees frequently contains phenolic glycosides, and seed kernels amygdalin, which releases hydrocyanic acid on hydrolysis.

It appears probable also that the glycosides may be storage depots for sugars required for quick release at some stage of development. Many plant colouring matters are of glycosidic nature, for example the anthocyanins and the flavone glycosides, whilst dyestuffs such as indigo and alizarin were formerly obtained exclusively by the hydrolysis of the glycosides indican and ruberythric acid respectively.

Phenolic Glycosides

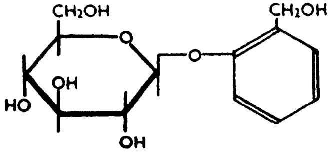
Arbutin, found in the leaves of the Bear-berry (*Arbutus uva-ursi*) and in leaves of some species of *Pyrus*, more particularly many varieties of pear, is hydroquinone-β-D-glucoside.

Several syntheses have been described, such as that of Robertson and Waters* where benzoyl hydroquinone was condensed with acetobromoglucose in the presence of silver oxide and quinoline. After deacylation with ammonia, arbutin was obtained. Along with arbutin, methyl arbutin is frequently found. The yellow colour of certain fallen leaves is due to the oxidation of this material, whilst the black colour of others is due to the liberation from arbutin of hydroquinone which is readily oxidised by plant oxidising enzymes.

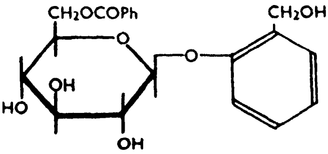
---

* *J.C.S.*, 1930, 2729.

Salicin, an important glycoside of willow bark, is saligenin-β-D-glucoside. Oxidation with nitric acid gives helicin, the salicylaldehyde glucoside which is, however, not found in nature. When salicin is benzoylated the natural glycoside, populin, is obtained; this is found in the bark of poplar trees. The benzoyl group in monobenzoyl salicin or populin is located in the sugar residue as shown and not in the salicyl alcohol, because oxidation of populin with nitric acid gives a benzoyl helicin.



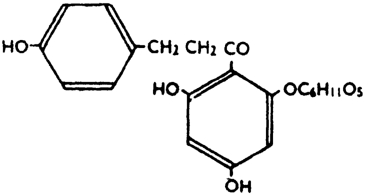
Salicin



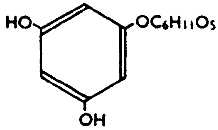
Populin

Gaultherin is the important glycoside associated with oil of wintergreen and is found in *Gaultheria procumbens* together with the appropriate enzyme, gaultherase, which converts it into glucose, xylose, and methyl salicylate. The sugar residue is primeverose in this instance.

Phloridzin is a complex glucoside found in the bark of plum and apple trees. The aglucone is phloretin (2,4,6-trihydroxy β-[4-hydroxyphenyl] propiophenone).



Phloridzin



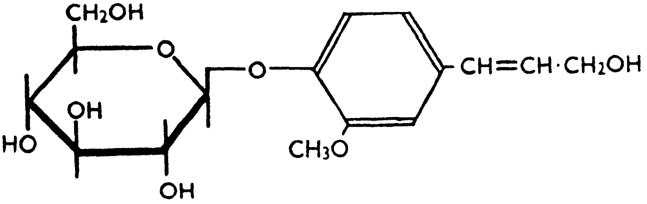
Phlorin

Treatment with barium hydroxide gives phenol propionic acid and phlorin, or phloroglucinol-β-D-glucoside, which has been synthesised from acetobromoglucose and sodium phloroglucide.[*] Phloridzin causes the so-called phloridzin diabetes when administered and is therefore of interest physiologically.

---

[*] Fischer and Strauss, *Ber.*, 1912, **45**, 2467.

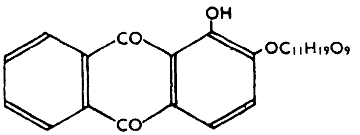
Coniferin and Syringin. Coniferyl alcohol β-D-glucoside is found in the cambial sap of pine trees, and syringin, the corresponding 5-methoxy compound, is the glucoside of Syringa.



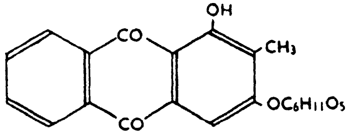
Coniferin

Hydroxy-anthraquinone Glycosides

Possibly the best known member of this class is *ruberythric acid,* the aglycone being alizarin and the sugar residue primeverose. The madder root from which alizarin was formerly obtained commercially



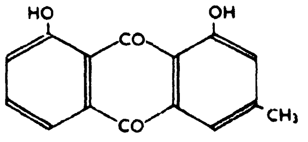
Ruberythric acid



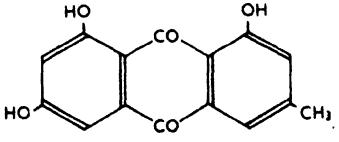
Rubiadin

contains other hydroxyanthraquinone glycosides namely purpurin and rubiadin ; the latter is found as the 3-glucoside of 1,3-dihydroxy 2-methyl anthraquinone.

Other anthraquinone glycosides which have applications in pharmacy as purgative drugs are found in rhubarb and aloes, for example chrysophanein of which the aglucone is chrysophanic acid and frangulin which is a frangula-emodin L-rhamnoside, found in Chinese rhubarb.



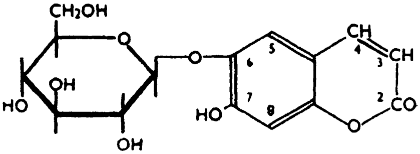
Chrysophanic acid



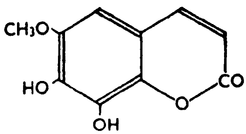
Frangula-emodin

Hydroxy-Coumarin Glycosides

Several glycosides are known which are derived from hydroxy-coumarins. Thus *Aesculin* found in the bark and buds of the horse chestnut tree (*Aesculus Hippocastanum*) is [6,7-dihydroxy-coumarin]-6-β-D-glucopyranoside.



Aesculin



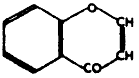
Fraxetin

In aqueous solution, especially in the presence of alkali, this substance gives a strongly marked blue fluorescence; aesculin is used occasionally as a protection against sunburn. The aglucone is called aesculetin.

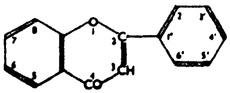
Daphnin, found in various species of *Daphne*, is [7,8-dihydroxy-coumarin]-7-β-D-glucoside and *Fraxin*, found in the bark of ash trees (*Fraxinus excelsior*) has an additional methoxy group on C_6 in the aglucone, fraxetin, the glucosidic residue being at position 8 in fraxin.

Hydroxy-Flavone Glycosides

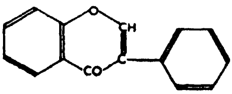
Among the colouring matter of plants the hydroxy-flavones are important, giving yellow and brown colours. Much of our present knowledge of this group is due to the work of Kostanecki who synthesised many of the aglycones. The substance flavone, which itself is found free as a white coating on flowers of many species of *Primula*, is derived from benzpyrone.



Benzpyrone

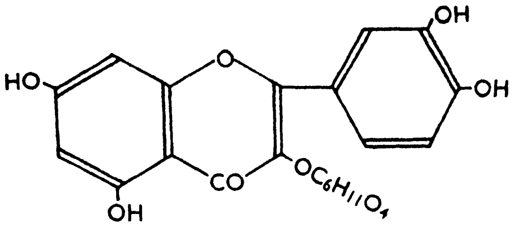


Flavone



*Iso*flavone

The most important member of this group is quercitrin which occurs in the American oak, *Quercus tinctoria*, and is used as a dye for silk and wool. It also occurs in yellow pansies, tea, and hops, and in many other plants. The aglycone, quercetin, is 3,5,7,3′,4′-penta-hydroxy-flavone and the aglycone has L-rhamnose condensed with the hydroxyl group on C_3.

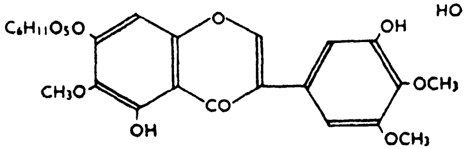


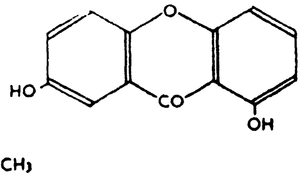
Quercitrin

It is frequently found in this group that the same aglucone is present in the same plant with the sugar residues attached to different hydroxyl groups. These substances have different absorption spectra and it has been suggested that the hydroxy flavone glycosides act as protective agents against the short waves of sunlight.

Rutin, quercetin-3-rutinoside, is assuming importance as a prophylactic measure for cases in which increased capillary fragility causes a risk of subcutaneous and submucous haemorrhages in diseases such as scurvy and diabetes. The best sources are the leaves and flowers of buckwheat (*Fagopyrum esculentum*), but rutin also occurs in forsythia, elder, tobacco and tomato stems. This type of activity shown by rutin was formerly ascribed to the glycoside, hesperidin (5,7,3′-trihydroxy 4′-methoxy flavanone-7-rutinoside) which for that reason had been designated vitamin P. It has recently been found, however, that rutin is more active than hesperidin in this connection.

Iridin which occurs in orris root (*Iris florentina*) might be mentioned as a member of the *iso*flavonol group, it is [5,7,3′-trihydroxy-6,4′,5′-trimethoxy *iso*flavone]-7-glucoside.*





Iridin

Euxanthone

Xanthone Pigments

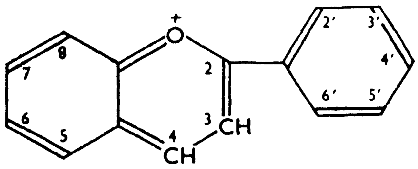
The xanthone glycosides are related to the flavone group. Euxanthic acid has been mentioned already (page 153); it is the 7-glucuronoside of 1,7-dihydroxy-xanthone or euxanthone.

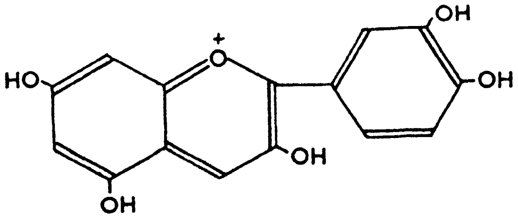
---

* W. Baker, *J.C.S.*, 1928, 1022.

The Anthocyanins

The brilliant blue and red colours of many flowers and berries are due to the glycosides of polyhydroxy phenyl pyrylium salts, called anthocyanins. The pioneer work of Willstätter and Everest* in this field was followed by the outstanding achievements of Sir Robert and Lady Robinson with the result that a great deal is known about the glycosides of this group. The glycosides are termed anthocyanins and the aglycones anthocyanidins. The nucleus of the aglycones is related to flavone, with the difference that the CO group at position 4 is reduced to CH. The oxygen atom is capable of oxonium salt formation (pyrylium salts) with acids and the cations resulting from this are red in colour. The phenolic hydroxyl groups which are present can form salts with bases also and the resulting anions are blue. The reader will have noted that the name anthocyanin has no connection with the cyanide radical; it so happens that the Greek root Kya'neos for blue appears in both words. Variations in the *p*H of the soil and the cell sap cause changes in the relative proportions of the various organic ions present, and consequently in the colour. Thus the same anthocyanin (cyanin) is found in the red rose and blue cornflower. Cyanidin, the aglycone, has hydroxyl groups on the 3,5,7,3′ and 4′ positions and cyanin is the corresponding 3,5,-β-diglucoside.





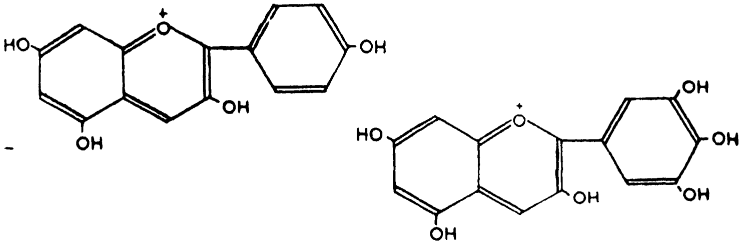
Cyanidin

---

* *Annalen*, 1913, **401**, 189.

The relationship of cyanidin to the flavonol, quercetin, is obvious, and Willstätter and Everest obtained cyanidin in poor yield by the reduction of quercetin with magnesium in methanolic hydrogen chloride.

Pelargonin, the colouring matter of the scarlet Geranium (*Pelargonium*), is the 4′-hydroxy derivative, and again has β-glucosidic groups on positions 3 and 5.



Pelargonidin Delphinidin

The cranberry contains *idaein* which is cyanidin 3-β-D-galactoside whereas *peonin* from the peony is cyanin 3′-methyl ether.

The aglucone delphinidin is the parent of another group and this is a hexahydroxy derivative. Thus *malvin*, a colouring matter from the grape and mallow, is delphinidin 3′5′-dimethyl ether 3,5-β-diglucopyranoside and *hirsutin* is the corresponding 3′,5′,7-trimethyl ether.

The constitution of the anthocyanidins has been worked out from a study of the effects of degradation with alkali. Thus pelargonidin on fusion with alkali gave phloroglucinol and *p*-hydroxybenzoic acid. Many anthocyanidins and anthocyanins have been synthesised by Robinson and his co-workers; as a typical example the synthesis of pelargonin by Robinson and Todd is appended.*

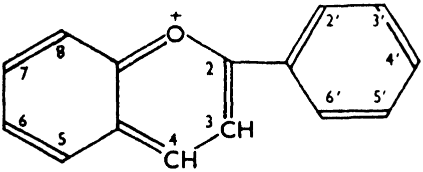
The condensation of phloroglucin aldehyde with acetobromoglucose gave 2-O-β-[glucosido]-phloroglucinaldehyde, which in ethyl acetate solution in the presence of hydrogen chloride condensed with ω-O-tetraacetyl β-glucosidoxy 4-acetoxy-acetophenone, to give after acetylation and acidification 7,4′-dihydroxy-3,5-di[β-glucosidoxy] flavylium chloride, that is pelargonin chloride, identical with the natural product (see page 178).

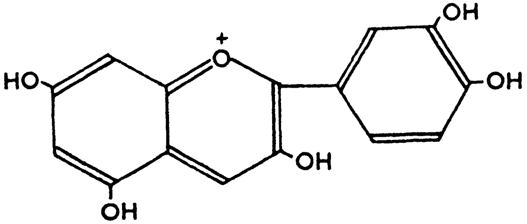
---

* *J.C.S.*, 1932, 2488.

The Anthocyanins

The brilliant blue and red colours of many flowers and berries are due to the glycosides of polyhydroxy phenyl pyrylium salts, called anthocyanins. The pioneer work of Willstätter and Everest* in this field was followed by the outstanding achievements of Sir Robert and Lady Robinson with the result that a great deal is known about the glycosides of this group. The glycosides are termed anthocyanins and the aglycones anthocyanidins. The nucleus of the aglycones is related to flavone, with the difference that the CO group at position 4 is reduced to CH. The oxygen atom is capable of oxonium salt formation (pyrylium salts) with acids and the cations resulting from this are red in colour. The phenolic hydroxyl groups which are present can form salts with bases also and the resulting anions are blue. The reader will have noted that the name anthocyanin has no connection with the cyanide radical; it so happens that the Greek root Kya'neos for blue appears in both words. Variations in the pH of the soil and the cell sap cause changes in the relative proportions of the various organic ions present, and consequently in the colour. Thus the same anthocyanin (cyanin) is found in the red rose and blue cornflower. Cyanidin, the aglycone, has hydroxyl groups on the 3,5,7,3′ and 4′ positions and cyanin is the corresponding 3,5,-β-diglucoside.





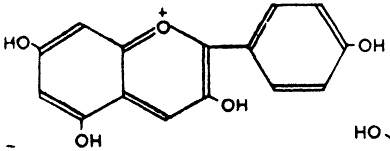
Cyanidin

---

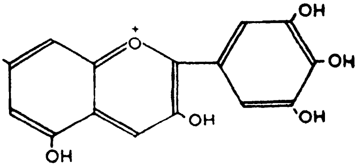
* *Annalen*, 1913, **401**, 189.

The relationship of cyanidin to the flavonol, quercetin, is obvious, and Willstätter and Everest obtained cyanidin in poor yield by the reduction of quercetin with magnesium in methanolic hydrogen chloride.

Pelargonin, the colouring matter of the scarlet Geranium (*Pelargonium*), is the 4′-hydroxy derivative, and again has β-glucosidic groups on positions 3 and 5.



Pelargonidin



Delphinidin

The cranberry contains *idaein* which is cyanidin 3-β-D-galactoside whereas *peonin* from the peony is cyanin 3′-methyl ether.

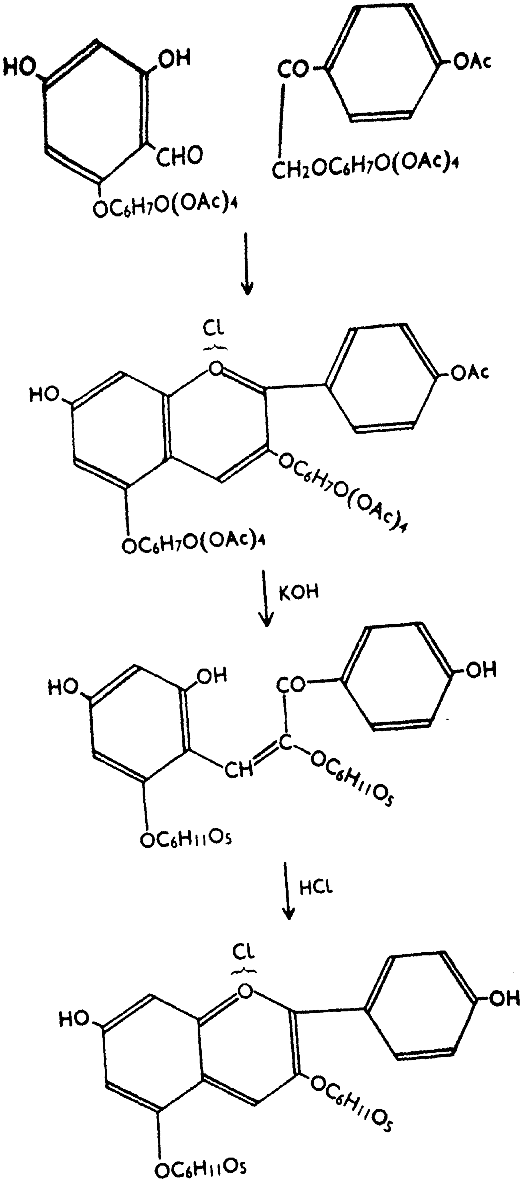
The aglucone delphinidin is the parent of another group and this is a hexahydroxy derivative. Thus *malvin*, a colouring matter from the grape and mallow, is delphinidin 3′5′-dimethyl ether 3,5-β-diglucopyranoside and *hirsutin* is the corresponding 3′,5′,7-trimethyl ether.

The constitution of the anthocyanidins has been worked out from a study of the effects of degradation with alkali. Thus pelargonidin on fusion with alkali gave phloroglucinol and *p*-hydroxybenzoic acid. Many anthocyanidins and anthocyanins have been synthesised by Robinson and his co-workers; as a typical example the synthesis of pelargonin by Robinson and Todd is appended.*

The condensation of phloroglucin aldehyde with acetobromoglucose gave 2-O-β-[glucosido]-phloroglucinaldehyde, which in ethyl acetate solution in the presence of hydrogen chloride condensed with ω-O-tetraacetyl β-glucosidoxy 4-acetoxy-acetophenone, to give after acetylation and acidification 7,4′-dihydroxy-3,5-di[β-glucosidoxy] flavylium chloride, that is pelargonin chloride, identical with the natural product (see page 178).

---

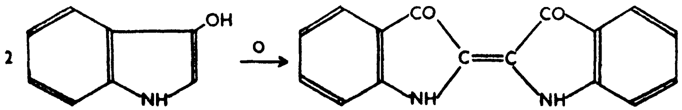
* *J.C.S.*, 1932, 2488.



Pelargonin chloride

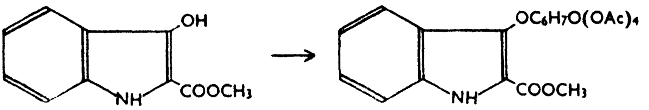
Indican

The glucoside indican is found in plants of the genus *Indigofera* and was formerly of great importance in the preparation of the vat dyestuff indigo. The aglucone is indoxyl or 3-hydroxy indole and it is set free together with a molecule of glucose by an enzyme present in the plant. On atmospheric oxidation indoxyl is transformed into indigotin, the insoluble colouring matter



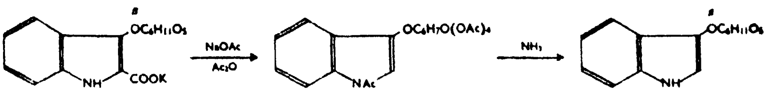
Indoxyl Indigotin

Indican has been synthesised as follows*: Methyl 3-hydroxyindole 2-carboxylate (I) was condensed with acetobromoglucose in acetone in the presence of potassium hydroxide to give methyl 3-O-tetra-acetyl β-glucosidoxyindole 2-carboxylate (II). By treatment with methanolic potash 3-β-glucosidoxyindole 2-carboxylic acid (III) was



I II

isolated as the potassium salt. Fusion with sodium acetate and acetic anhydride eliminated the carboxyl group to give l-acetyl 3-O-tetraacetyl β-glucosidoxyindole (IV) which on deacetylation with ammonia gave 3-β-glucosidoxyindole identical with natural indican (V).



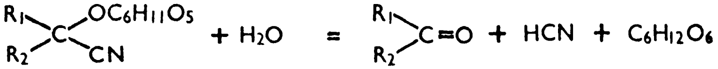
III IV V

---

* Robertson and Waters, *J.C.S.*, 1933, 30; Robertson, *J.C.S.*, 1927, 1937.

The Cyanogenetic Glycosides

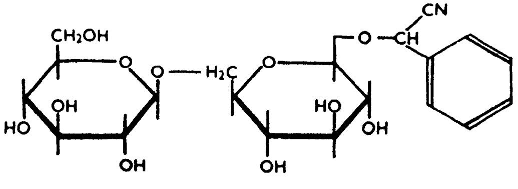
Many plants and seeds liberate hydrocyanic acid when the leaves or seeds are bruised; this is due to the decomposition of glycosides containing this substance; the best known example of the group of cyanogenetic glycosides is amygdalin. These glycosides have cyanohydrins as the aglycone which are decomposed by hydrolysis into the parent aldehyde or ketone and hydrocyanic acid. When R_1 and R_2 are different or $R_2 = H$, we have an asymmetric centre in the aglycone portion.



Amygdalin

The crystalline glycoside amygdalin was first isolated in 1830 by Robiquet and Boutron-Charlard, and Liebig and Wöhler showed that it was hydrolysed by emulsin to give two molecules of glucose, benzaldehyde and hydrocyanic acid. Later workers found that hydrolysis could be effected by acids and Fischer showed that by the action of yeast extract, a new glucoside, mandelonitrile glucoside, was obtained together with a molecule of glucose. Evidently, therefore, since only one hydroxyl group was available, a disaccharide was present in amygdalin and it was proved to be gentiobiose by Haworth and Leitch.*

Synthesis† proved amygdalin to be D(–)-mandelonitrile-β-gentiobioside.



Amygdalin

---

* *J.C.S.*, 1922, 1921.

† Campbell and Haworth, *J.C.S.*, 1924, 1337.

Acetobromogentiobiose + Ethyl DL-mandelate (I)

↓ Ag_2O

Ethyl DL-heptaacetyl amygdalinate (II)

CH_3OH ↓ NH_3

DL-Amygdalinamide (III)

Pyridine ↓ Ac_2O

DL-Heptaacetyl amygdalinamide (IV)
(resolved by fractional crystallisation
of the pyridine compound)

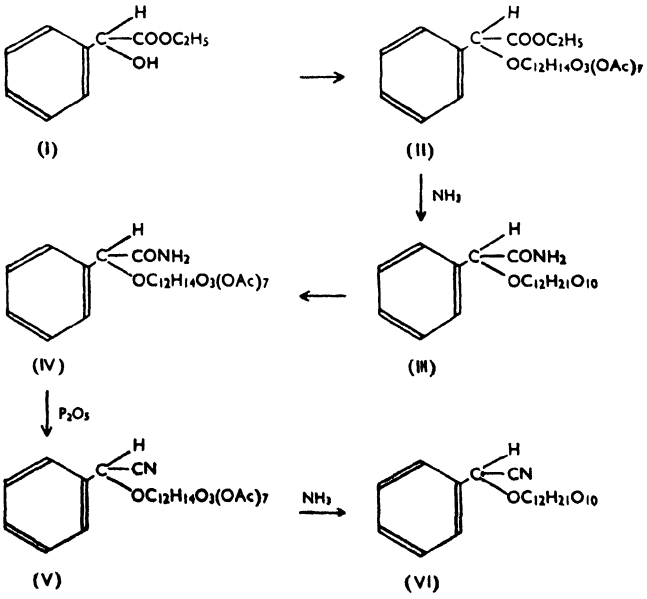
P_2O_5 ↓

D-Heptaacetyl amygdalin (V)

CH_3OH ↓ NH_3

Amygdalin (VI)

Synthesis of Amygdalin



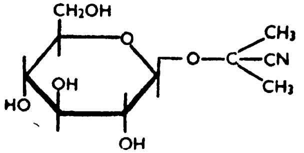
Both the final product and the heptaacetyl amygdalin were identical with natural amygdalin and its heptaacetate. Amygdalin is therefore D(–)-mandelonitrile β-gentiobioside. [The (–) refers to the sign of rotation of the naturally occurring D(–)-mandelic acid. The corresponding nitrile is actually dextrorotatory.]

The glucoside called by Fischer mandelonitrile glucoside and obtained by the action of yeast extract on amygdalin is found in some plants of the *Prunus spp.* such as in the bark of the wild cherry and is called *prunasin.* *Vicianin* a glycoside isolated from wild vetch seeds (*Vicia angustifolia*) is the analogue of amygdalin with the disaccharide vicianose (page 87) replacing gentiobiose.

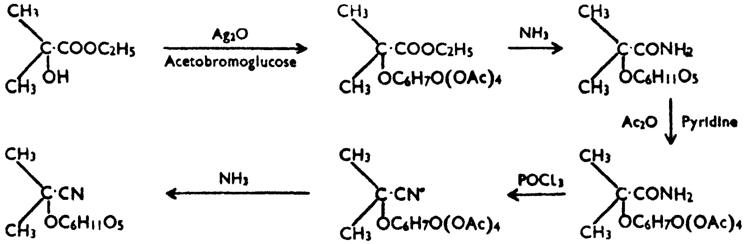
The common elder (*Sambucus niger*) leaves yield a glycoside called *sambunigrin* which is L(+)-mandelonitrile β-D-glucoside and is thus a diastereoisomer of *prunasin.* Fischer and Bergmann[*] synthesised prunasin, sambunigrin and the racemic DL-mandelonitrile β-D-glucoside (*prulaurasin*) by steps similar to those already described for amygdalin.

Linamarin

The aglucone of the cyanogenetic glucoside linamarin, found in flax plants is acetone cyanhydrin. This glycoside is also contained in the seeds of the rubber tree (*Hevea brasiliensis*). Synthesis followed the steps indicated below.[†]



Linamarin



α-Hydroxy*iso*butyric ester was condensed with acetobromoglucose, the product converted into an amide with ammonia, and finally into the cyanide.

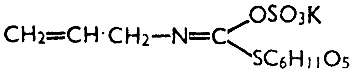
---

[*] *Ber.*, 1917, **50**, 1047.

[†] Fischer and Anger, *Ber.*, 1919, **52**, 854.

The Mustard Oil Glycosides

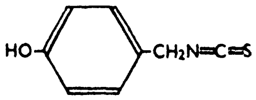
Glycosides in plants of the family *Cruciferae* contain sulphur, and when the seeds are bruised in the presence of water, pungent mustard oils are liberated. Thus black mustard seed (*Sinapis nigra*) gives rise to allyl *iso*thiocyanate $CH_2=CH-CH_2-N=C=S$, glucose and potassium hydrogen sulphate by the action of water in the presence of the enzyme myrosinase. The glycoside is called *sinigrin* and its constitution, according to Gadamer (1897) and substantiated by W. S. Schneider and Wrede* is given as



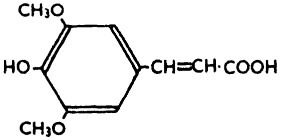
$$CH_2{=}CH\cdot CH_2{-}N{=}C\begin{matrix}\nearrow OSO_3K\\ \searrow SC_6H_{11}O_5\end{matrix}$$

so that it is really a derivative of thioglucose. Sinigrin is not hydrolysed either by emulsin or yeast extract. By treatment with potassium methoxide, thioglucose and potassium sulphate may be obtained, whereas ammoniacal silver nitrate gives the silver salt of thioglucose.

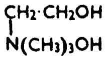
White mustard seed contains a more complex glycoside called *sinalbin* which is hydrolysed also by myrosinase to *p*-hydroxybenzyl *iso*thiocyanate (I), glucose and sinapin sulphate. This latter substance with baryta gives sinapinic acid (II) and choline (III).



(I)

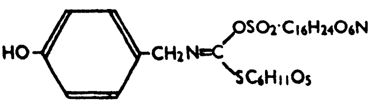


(II)



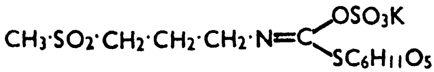
$$\begin{matrix}CH_2\cdot CH_2OH\\ |\\ N(CH_3)_3OH\end{matrix}$$

(III)



Sinalbin

Wallflower seeds contain glucocheirolin, which again is hydrolysed by myrosinase to potassium hydrogen sulphate, glucose and cheirolin – γ-methylsulphone propyl*iso*thiocyanate. The glucoside is therefore



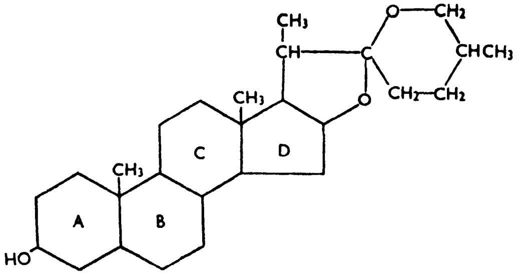
$$CH_3\cdot SO_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot N{=}C\begin{matrix}\nearrow OSO_3K\\ \searrow SC_6H_{11}O_5\end{matrix}$$

β-Phenyl ethyl *iso*thiocyanate $C_6H_5CH_2CH_2NCS$ is produced on the hydrolysis of the glycoside in nasturtiums and turnips.

[*] *Ber.*, 1914, **47**, 2225.

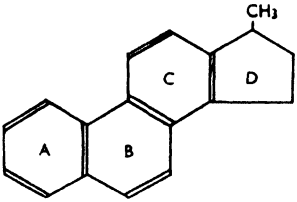
Glycosides Derived from *Cyclo*pentenophenanthrene

Saponins. There is a large group of glycosides called saponins which give a soapy foam on mixing with water, and are very toxic. Small quantities are lethal to cold blooded animals such as fish, and plants containing saponins are used as a means of catching fish by some natives of Brazil. In red blooded animals saponins cause a haemolysis or a disintegration of the red blood cells. By hydrolysis of a saponin with acid the aglycone is obtained; this is called a sapogenin. Thus the saponin, *parillin* or *sarsasaponin* from sarsaparilla root on hydrolysis gives rhamnose, glucose and sarsasapogenin for which the formula below has been suggested.* This sapogenin on



Sarsasapogenin

dehydrogenation with selenium according to Diels gives methyl *cyclo*pentenophenanthrene.



Methyl *cyclo*pentenophenanthrene

A number of saponins for example, *digitonin* and *gitonin* belong to this group, which is related to the sterols. A second group of saponins is even more complex having a triterpenoid nucleus and giving 1,2,7-trimethyl naphthalene (sapotalene) on dehydrogenation.

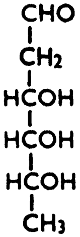
The Cardiac Glucosides

Certain glycosides also related to the sterols and isolated from

---

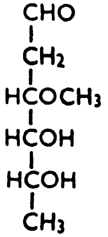
* Marker and Rohrmann, *J. Amer. Chem. Soc.*, 1939, **61**, 846.

Digitalis and *Strophanthus spp.* are valuable in medicine for stimulating the heart muscle in cases of disease, although in larger quantities they are very toxic. It is noteworthy that the sugars in these glycosides are usually deoxy sugars, such as digitoxose, cymarose and digitalose.



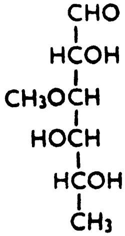
$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{CH_2} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{CH_3} \end{array}$$

D-Digitoxose



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{CH_2} \\ | \\ \mathrm{HCOCH_3} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{CH_3} \end{array}$$

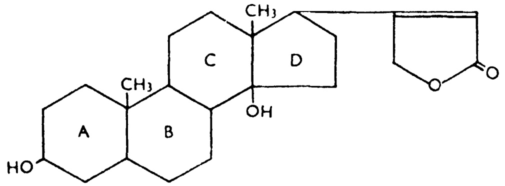
Cymarose



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{CH_3OCH} \\ | \\ \mathrm{HOCH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{CH_3} \end{array}$$

Digitalose
(3-methyl D-fucose)

Thus digitoxin gives on hydrolysis digitoxigenin and three molecules of digitoxose.

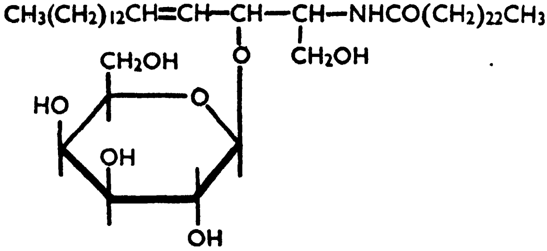


Digitoxigenin

It will be noted that the aglycones of this group which are closely related to the toad poisons have a sterol skeleton and a side chain which is an unsaturated lactone. In the glycosides the hydroxyl group in position 3 is the one linked to the sugar residues.

Miscellaneous Complex Glycosides

It is only possible to mention in passing certain other complex glycosides. Among these are the solanaceous alkaloids such as solanine from potatoes and deadly nightshade, which on hydrolysis gives solanidine, glucose, rhamnose and galactose. The nerve tissues and brains of animals contain compounds called cerebrosides. The amino alcohol, sphingosine $CH_3(CH_2)_{12}CH = CH.CH(OH)CH(NH_2)CH_2OH$ is present combined glycosidically through the secondary hydroxyl group with D-galactose and through the amino group with long chain fatty acids such as lignoceric acid $CH_3(CH_2)_{22}COOH$ and cerebronic acid $CH_3(CH_2)_{21}CHOH.COOH$.

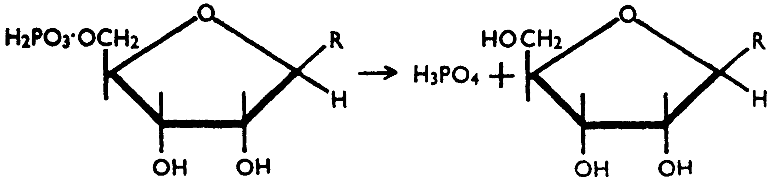


A Cerebroside

Nitrogen Glycosides

Glycosides in which the union to the aglycone is made through nitrogen are of great importance since the nucleic acids belong to this group.

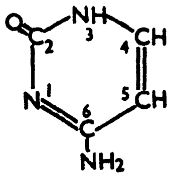
The nucleoproteins contained in all living cells are made up of a nucleic acid portion and a protein portion. The nucleic acid is composed of four nucleotide building units, and these are nitrogen glycosides of pyrimidines or purines which are esterified on the sugar residue (which may be D-ribose or 2-deoxy-D-ribose) with phosphoric acid. The mononucleotides may be divested of their phosphate residue by heating with water or mild alkali, and *nucleosides* are then produced. Thus muscle adenylic acid gives adenosine.



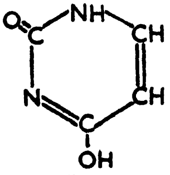
Muscle Adenylic Acid Adenosine R = Adenyl

The aglycones in these nucleosides are either pyrimidines such as cytosine and uracil, or purines such as adenine and guanine. As examples of these substances the pyrimidine nucleosides, cytidine and uridine, and the purine nucleoside adenosine will be considered.

Cytidine and Uridine. The pyrimidine (aglycone) portions of cytidine and uridine are cytosine and uracil respectively.

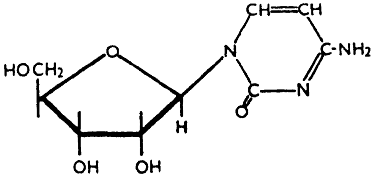


Cytosine

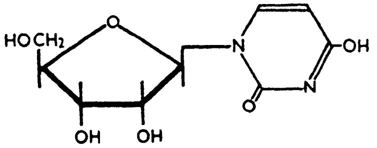


Uracil

Cytidine is readily converted to uridine by deamination, and the methylation studies of Levene and Tipson* showed that in uridine and cytidine the ribose residue was furanose and the point of union at position 3 of the base.

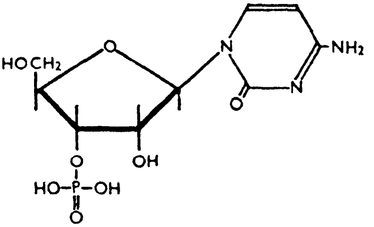


Cytidine



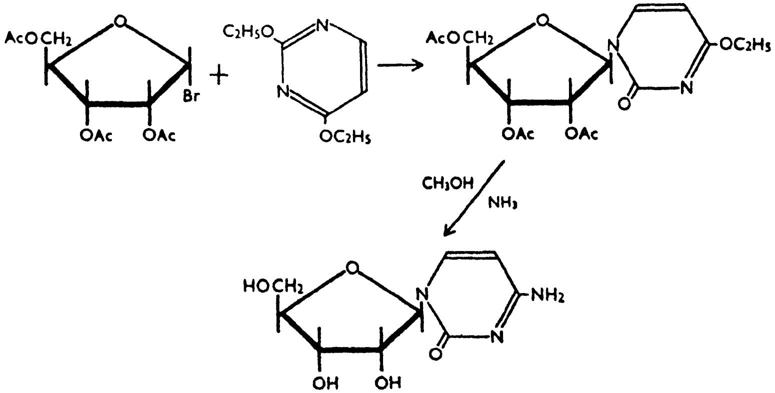
Uridine

The corresponding mononucleotides cytidylic and uridylic acids carry an ethereal phosphate residue on C_3 of the ribofuranose unit.



Cytidylic acid

The synthesis of cytidine has been achieved by Howard, Lythgoe and Todd† as follows: 1,2,3-Triacetyl 5-trityl D-ribofuranose was converted by hydrogenolysis to 1,2,3-triacetyl D-ribofuranose which gave crystalline tetraacetyl D-ribofuranose on acetylation. The corresponding 1-bromo derivative was then condensed with 2,6-diethoxypyrimidine to give triacetyl 3-[β-ribofuranosido]-6-ethoxypyrimidine which on treatment with methanolic ammonia gave cytidine isolated as the sulphate and found to be identical with the sulphate prepared from the natural product.

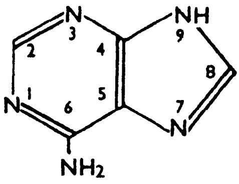


---

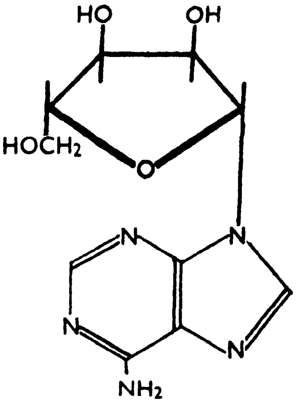
* *J. Biol. Chem.*, 1933, **101**, 529; 1934, **104**, 385, **105**, 419; Tipson, see *Advances in Carbohydrate Chemistry*, 1945, **1**, 193.

† *J.C.S.*, 1947, 1052.

Adenosine. The purine base associated with D-ribose in adenosine is adenine.



Adenine

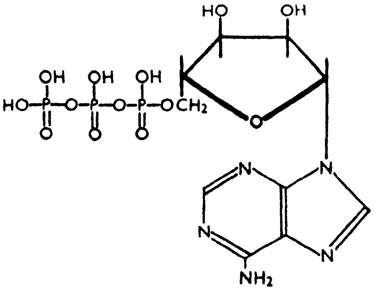


Adenosine

The ribofuranose residue is attached to position 9 of the adenine residue.*

The adenylic acid of muscle is the 5-phosphate whilst the adenylic acid isolated from yeast is the 3-phosphate.

Adenosine triphosphate is of great importance in enzymic phosphorylation processes (see page 138) for it can convert glucose into glucose 6-phosphate; it plays a part in muscle glycolysis, and alcoholic fermentation, its activity being due to the presence of a so-called energy rich phosphate bond.

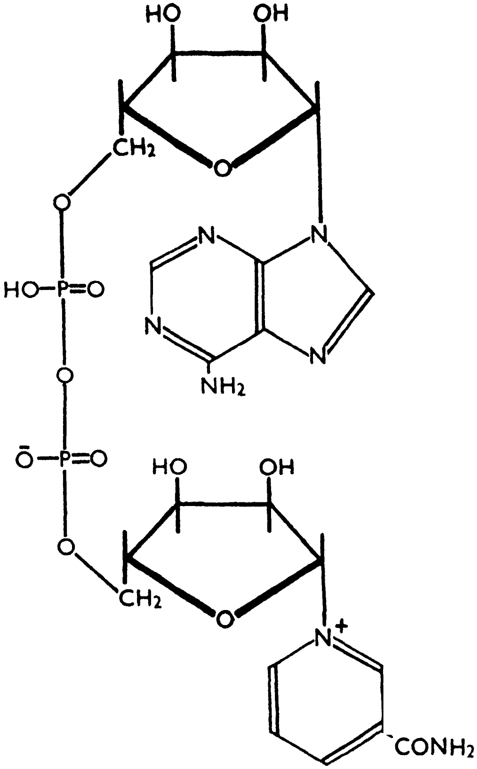


Adenosine triphosphate

Adenylic acid also enters into the structure of coenzyme I which acts as a hydrogen carrier in alcoholic fermentation. This substance is a dinucleotide of adenylic acid and the nicotinic acid amide nucleotide.

---

* Gulland, *J.C.S.*, 1944, 208; Todd, *J.C.S.*, 1946, 647.

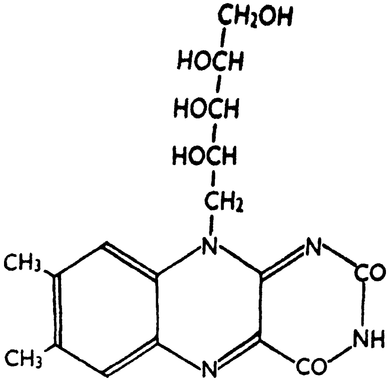


Coenzyme I

The nicotinamide portion acts as the hydrogen carrier being capable of taking up two atoms of hydrogen. Coenzyme II has a similar structure except that three atoms of phosphoric acid are interposed between the two nucleoside residues.

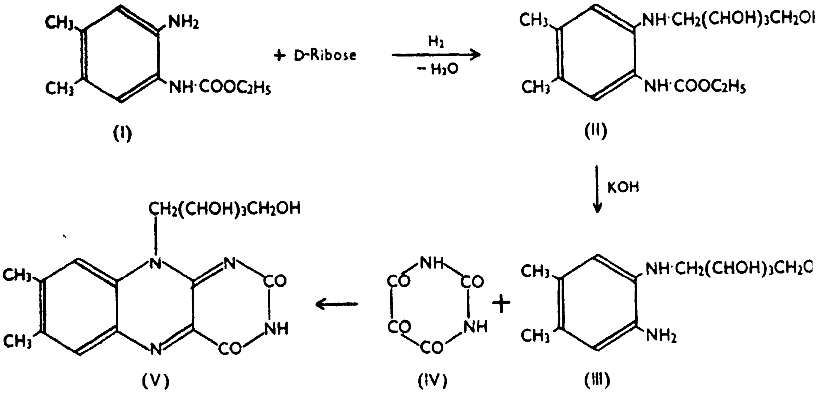
Riboflavin (Lactoflavin)

Although not in actual fact a glycoside, the so-called lactoflavin (Vitamin B_2) found in milk as a growth factor has points of similarity with the compounds mentioned above. Riboflavin is a derivative of ribitol (adonitol), the reduction product of ribose and is 6,7-dimethyl 9-[D-ribityl]-*iso*alloxazine.



Riboflavin

The steps in a total synthesis of this substance* are indicated below.



An equimolecular mixture of D-ribose and 1-amino-2-carbethoxyamino 4,5-dimethyl benzene (I) was hydrogenated to give 2-carbethoxyamino 4,5-dimethyl phenyl ribamine (II). Hydrolysis with alkali gave the corresponding 2-amino derivative (III) which on condensation with alloxan (IV) in acid solution gave lactoflavin (V).

* Karrer *et al.*, *Helv. Chim. Acta*, 1935, **18**, 69, 522.

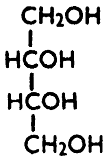
CHAPTER X

OTHER IMPORTANT NATURAL PRODUCTS

The Polyhydric Alcohols

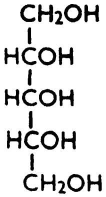
ALL reducing sugars can be converted into polyhydric alcohols by reduction, but several members of this class are of special interest since they occur in nature.

Erythritol, a tetrahydric alcohol, is found in algae, fungi and lichens, occurring in the latter as erythrin, an ester of orsellinic acid. Erythritol is a *meso* form



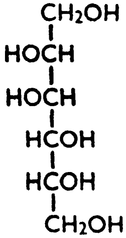
$$\begin{array}{c} CH_2OH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

Erythritol



$$\begin{array}{c} CH_2OH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

Adonitol



$$\begin{array}{c} CH_2OH \\ | \\ HOCH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

D-Mannitol

The pentahydric alcohol, *adonitol* occurs in *Adonis vernalis* and is also a *meso* form, it may be obtained by the reduction of ribose.

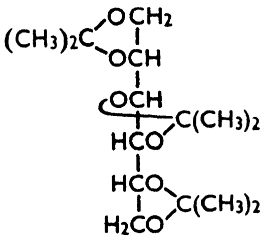
Hexahydric Alcohols

Four hexahydric alcohols have been found so far in nature, D-mannitol, D-sorbitol, dulcitol and L-iditol.

D-*Mannitol*

Mannitol is found in the manna of the ash tree and in many other plants; it is especially abundant in the brown seaweeds, quantities of over 37% of the dry weight having been found in July 1947 in *Laminaria digitata* (growing at a depth of 5 fathoms). The seasonal variations of mannitol in seaweeds are quite remarkable, the quantity present in the fronds of *L. digitata* (from a sea loch) rising from about 3% in December 1945 to 25% in June 1946. Mannitol, together with sorbitol, is produced when sucrose is hydrogenated under pressure, so that sucrose also represents a potential source of this material.

Like other polyhydric alcohols, mannitol increases the acidity of solutions of boric acid, making possible direct titration to phenolphthalein and the almost negligible specific rotation of mannitol solutions is greatly enhanced by the addition of borates, arsenates and other inorganic salts. Mannitol forms esters with acids; mannitol hexanitrate is a powerful explosive although it has not been used on a large scale in this country, presumably owing to the present high cost of the starting material. Mannitol has been used, however, together with glycerol in the preparation of synthetic resins of the " glyptal " type, the six hydroxyl groups increasing the probability of the formation of cross linkages. The six hydroxyl groups are not esterified simultaneously, however, for, depending on the conditions, mannitol dibenzoates, tetrabenzoates and hexabenzoates have been isolated. Condensation with carbonyl compounds such as aldehydes and ketones takes place even more readily with these polyhydric alcohols than with sugars. As an example of this type of reaction product tribenzylidene mannitol may be mentioned; it is made by shaking together mannitol, concentrated hydrochloric acid and benzaldehyde. Triacetone mannitol has been shown to be the 1,2-3,4-5,6-triacetone derivative.* Since there is no ring structure, and " free rotation " may take place between the carbon atoms, the condition that *cis*-hydroxyl groups are necessary for condensation does not apply in this case.


Triacetone mannitol

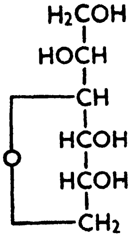
By heating mannitol in a vacuum a mixture of anhydrides may be isolated. Dehydration with formic acid yields a 3,6-anhydride (which is of course here the same as a 1,4-anhydride), D-mannitan, and a 1,4-3,6-dianhydride called *iso*mannide;† this latter substance is best prepared by heating mannitol with concentrated hydrochloric acid, while treatment of mannitol dichlorhydrin with sodium amalgam gives another dianhydride called β-mannide.‡ Another isomer, *neo*-mannide, has been shown to be 1,5-3,6-dianhydro mannitol§ and is prepared conveniently from 6-tosyl 2,3,4-tribenzoyl styracitol by deacylation. Styracitol, a naturally occurring monoanhydro man-

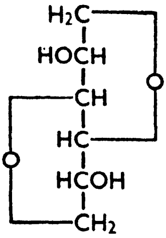
* Wiggins, *J.C.S.*, 1946, 13.

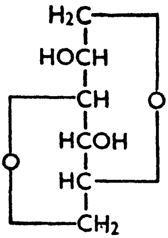
† Wiggins, *J.C.S.*, 1945, 4; 1948, 2204.

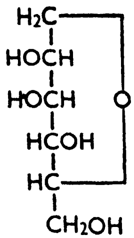
‡ Siwoloboff, *Annalen*, 1886, **233**, 368.

§ Hockett and Sheffield, *J. Amer. Chem. Soc.*, 1946, **68**, 937.


D-Mannitan


*Iso*mannide


*Neo*mannide


Styracitol

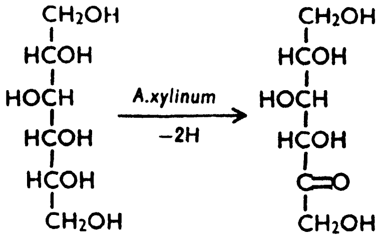
nitol found in *Styrax obassia*, has been shown to be 1,5-anhydro D-mannitol,* and may be isolated along with 1,4-anhydro mannitol (mannitan) when mannitol is dehydrated.

Mannitol on oxidation with nitric acid gives D-mannose (page 13), D-mannonic acid, D-mannosaccharic acid, and other oxidation products depending on the conditions. When treated with Bertrand's sorbose bacterium (*acetobacter xylinum*), D-fructose is produced.

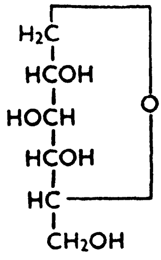
D-*Sorbitol*

The important polyhydric alcohol related to D-glucose is found in nature in many fruits but especially in the fruit of the mountain ash or rowan tree, where it occurs together with L-sorbose and L-iditol.

Commercially sorbitol is obtained from D-glucose by electrolytic reduction or by catalytic hydrogenation. Its chief use is in the preparation of L-sorbose, an intermediate in the manufacture of ascorbic acid (Vitamin C). This oxidation is accomplished by Bertrand's sorbose bacterium.



D-Sorbitol L-Sorbose

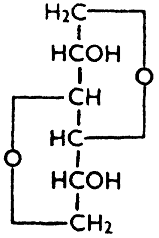

Polygalitol

This raises the question whether sorbitol should be assigned to the D or the L series. Note that this difficulty does not arise with mannitol because of its peculiar symmetry. It appears that the historical relationship of sorbitol to D-glucose must take precedence over the discovery of its relationship to L-sorbose* and although there is no fundamental scientific foundation for such a decision, ordinary sorbitol is designated D-sorbitol. Sorbitol forms esters and condensation products with aldehydes and ketones in the same way as mannitol.

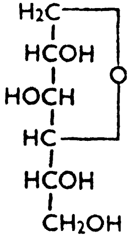
---

* Richtmyer, Carr and Hudson, *J. Amer. Chem. Soc.*, 1943, 65, 1477.

Polygalitol, an epimer of styracitol occuring in *Polygala amara*, has been shown by synthesis to be 1,5-anhydro sorbitol† (1,5-sorbitan) and other anhydrides such as arlitan and *iso*sorbide are known. The latter is readily obtained by boiling sorbitol with concentrated hydrochloric acid for 24 hours, and has been shown to be 1,4-3,6-dianhydro sorbitol.‡ Arlitan (1,4-sorbitan) is obtained along with polygalitol when sorbitol is dehydrated.§



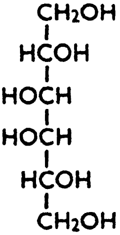
Isosorbide



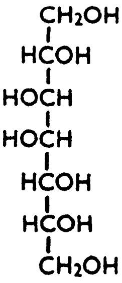
Arlitan

Dulcitol

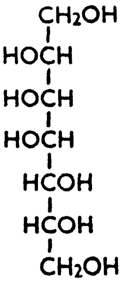
The reduction product of D-galactose, which finds uses in bacteriology, is the inactive hexahydric alcohol dulcitol, discovered in



Dulcitol



D-Perseitol



D-Volemitol

---

* Hudson, *Advances in Carbohydrate Chemistry*, 1945, **1**, 1, Academic Press, New York.

† Richtmyer, Carr and Hudson, *J. Amer. Chem. Soc.*, 1943, **65**, 1477.

‡ Montgomery and Wiggins, *J.C.S.*, 1946, 390.

§ Goepp, Soltzberg and W. Freudenberg, *J. Amer. Chem. Soc.*, 1946, **68**, 919.

Madagascar manna by A. Laurent in 1850 and since found in a variety of plants.

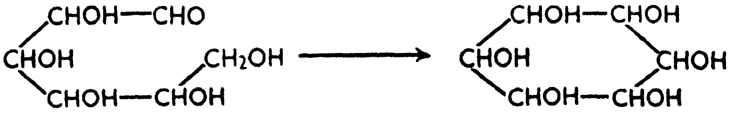
Heptahydric Alcohols

Two heptahydric alcohols have been found in nature, D-perseitol (D-manno-D-*gala*heptitol) in avocado fruit and D-volemitol (D-manno-D-*talo*heptitol) in the fungus *Lactarius volemus*, and in the roots of some species of *Primula*.

Cyclic Polyhydric Alcohols

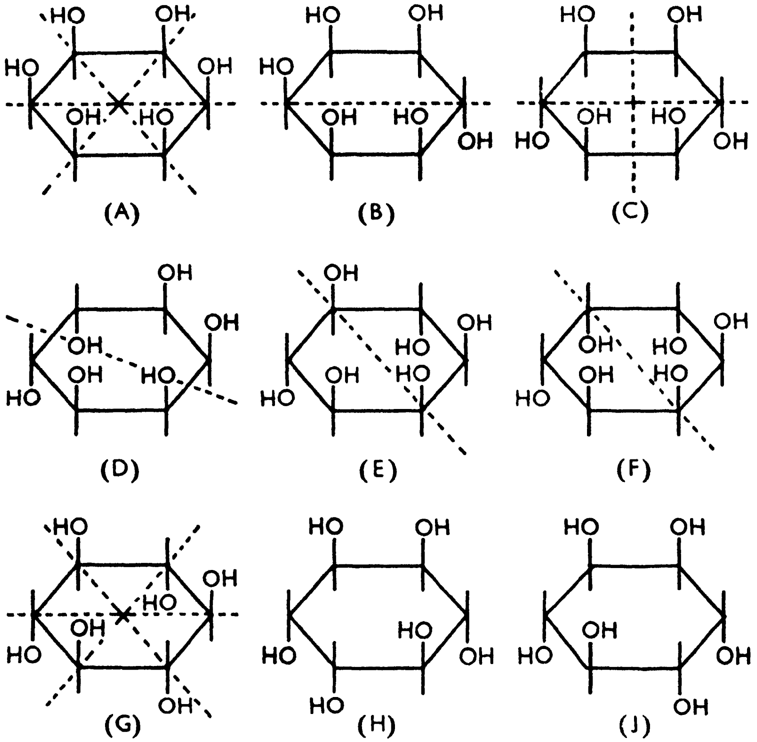
The Inositols

Certain hexahydroxy *cyclo*hexanes called the inositols occur both in plants and animals. These crystalline substances have a sweet taste, are soluble in water, but are non-reducing. Whether they are formed from aldohexoses by an aldol condensation involving C_1 and C_6 may be doubtful, but they are either end products derived from sugars or act as intermediaries in sugar interconversions in some way as yet unknown. It is also not improbable that the inositols are links between the sugars and the phenols; in theory, at any rate, the loss of three molecules of water from inositol could give phloroglucinol.



The stereochemistry of the inositols is very interesting, if at first sight, somewhat confusing, since although there are two optically active inositols, neither possesses an asymmetric carbon atom as such. It is possible to write down the formulae of eight stereoisomers, one of which is capable of representation in mirror image forms because it has no plane or centre of symmetry. In the accompanying formulae the directions of the planes of symmetry are indicated by dotted lines.

The Inositols

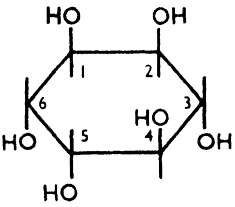


The Active Inositols. Racemic inositol was found in ripe mistletoe berries by Tanret; the active forms occur most plentifully as the monomethyl ethers. *d*-Inositol monomethyl ether or pinitol is found in the resin of the Californian pine (*Pinus lambertiana*) and in the senna plant, and *l*-inositol methyl ether or quebrachitol is found in quebracho bark. By demethylation with hydrogen iodide the corresponding active inositols may be obtained.

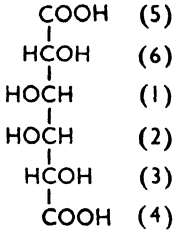
The constitution of *l*-inositol (H) has been demonstrated* by oxidation with permanganate, mucic acid and D-saccharic acid being produced, the former by breaking the ring between C_4 and C_5 and the latter by scission between C_1 and C_2, C_5 and C_6.

---

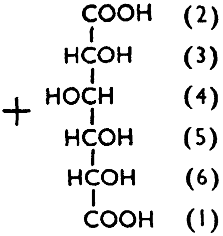
* Posternak, *Helv. Chim. Acta*, 1936, **19**, 1007.



l-Inositol



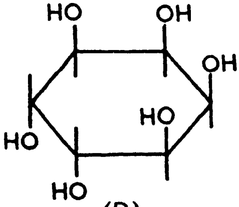
Mucic acid



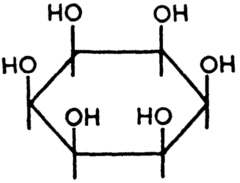
D-Saccharic acid

*Meso**Inositol.* The so-called *meso*inositol, the most important of the inositols, is the substance known as Bios I, a growth factor which stimulates yeast production. Acting alone it has very little effect but, in conjunction with biotin (bios II), yeast propagation is greatly stimulated. *Meso*inositol is widely distributed both in plants and animals, being found in asparagus leaves, oak-, ash-, walnut-bark, in the grape vine and in fungi. The compound known as phytic acid or phytin is *meso*inositol hexaphosphate.*

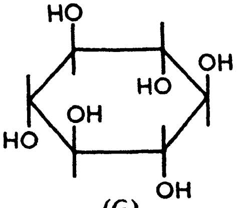
The determination of the structure of *meso*inositol has been a matter of great difficulty. A solution has now been provided by the work of T. Posternak.† By the action of a phosphatase on phytin an optically active tetraphosphate and an inactive monophosphate was obtained. The isolation of this latter substance excluded formulation D, as a possibility, since any monosubstituted derivative of this isomer must be resolvable; A is excluded by the isolation of the optically active tetraphosphate since even with the most unsymmetrical substitution the product would still have a plane of symmetry. A mixture of *meso*inositol mono- and diphosphates on oxidation with fuming nitric acid gave a mixture of racemic and *meso*tartaric acids. This excludes G which could give



(D)



(A)



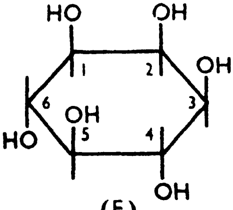
(G)

---

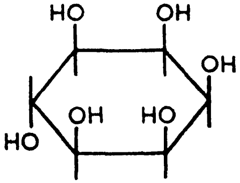
* Winterstein, *Z. physiol. Chem.*, 1908, **58**, 118.

† *Helv. Chim. Acta*, 1935, **18**, 1283; 1929, **12**, 1165; 1942, **25**, 746; see Jones, *Ann. Reports Chem. Soc.*, 1946, 167.

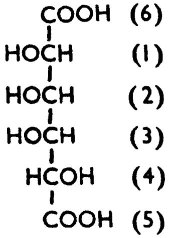
no *meso*tartaric acid on oxidation. When *meso*inositol was oxidised with alkaline permanganate complex racemic mixtures containing both D- and L-talomucic and D- and L-saccharic acids were isolated, which could only arise from E and B of the remaining possibilities. Thus from E by oxidation between C_5 and C_6, D-talomucic acid (I), between C_4 and C_5, L-talomucic acid (II), between C_1 and C_6, D-saccharic acid (III) and between C_3 and C_4 L-saccharic acid (IV) could arise.



(E)

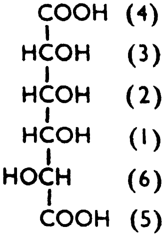


(B)



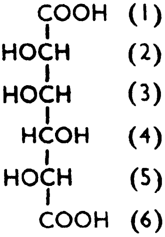
$$\begin{array}{ll} \mathrm{COOH} & (6)\\ \mathrm{HOCH} & (1)\\ \mathrm{HOCH} & (2)\\ \mathrm{HOCH} & (3)\\ \mathrm{HCOH} & (4)\\ \mathrm{COOH} & (5) \end{array}$$

(I)



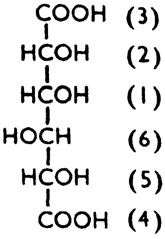
$$\begin{array}{ll} \mathrm{COOH} & (4)\\ \mathrm{HCOH} & (3)\\ \mathrm{HCOH} & (2)\\ \mathrm{HCOH} & (1)\\ \mathrm{HOCH} & (6)\\ \mathrm{COOH} & (5) \end{array}$$

(II)



$$\begin{array}{ll} \mathrm{COOH} & (1)\\ \mathrm{HOCH} & (2)\\ \mathrm{HOCH} & (3)\\ \mathrm{HCOH} & (4)\\ \mathrm{HOCH} & (5)\\ \mathrm{COOH} & (6) \end{array}$$

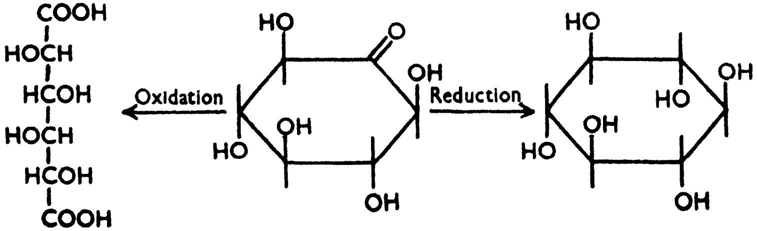
(III)



$$\begin{array}{ll} \mathrm{COOH} & (3)\\ \mathrm{HCOH} & (2)\\ \mathrm{HCOH} & (1)\\ \mathrm{HOCH} & (6)\\ \mathrm{HCOH} & (5)\\ \mathrm{COOH} & (4) \end{array}$$

(IV)

By the action of *Acetobacter suboxidans* on *meso*inositol, a ketose, *bio*-inosose, may be isolated,* and an examination of the products of the oxidation of this substance enabled Posternak to decide between the claims of E and B. Bio-inosose on oxidation gave DL-idosaccharic acid, and on reduction a mixture of *meso*inositol and another inactive



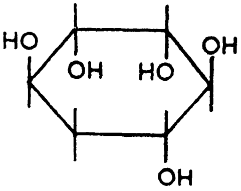
D-Idosaccharic acid Bio-inosose Scyllitol (G) inositol called *scyllitol* was obtained. Now a ketone derived from B could not give on oxidation the alternating arrangement of hydrogen and hydroxyl groups found in the idosaccharic acid; it follows therefore that *meso*inositol must be E and scyllitol G is the inositol with alternating hydrogen atoms and hydroxyl groups.

---

* Kluyver and Boezaardt, *Rec. trav. chim.*, 1939, **58**, 956.

Scyllitol. Hugo Müller* in his studies on inositols showed that an inactive cyclitol isolated from the leaves of the coconut palm (*Cocos nucifera*) and which is also found in acorns and the leaves of the shrub, *Helinus ovatus*, was the same as the substance scyllitol isolated much earlier from dog-fish liver and cartilage. This illustration serves to demonstrate the ubiquitous nature of the members of the inositol group.

Deoxy Inositols, Quercitols. Pentahydroxy *cyclo*hexanes also occur in plants. The best known is *d*-quercitol, $[\alpha]_D + 25{\cdot}6°$, found in acorns, and oak bark. This is represented as a deoxy derivative of active inositol and gives on oxidation with nitric acid, mucic acid and L-*arabo*trihydroxyglutaric acid. The compound called *l*-quercitol ($[\alpha]_D - 74°$) from *Gymnema sylvestre* is not the mirror image of *d*-quercitol, but another of the twelve theoretically possible optically active forms, ten different formulae being possible for this group of which six are unsymmetrical.



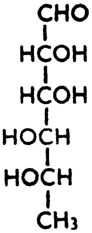
d-Quercitol

The Deoxy Sugars

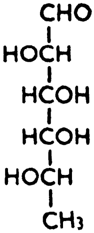
From time to time in this book deoxy or desoxy sugars have been mentioned, as for example in the reactions of glycals with mineral acids (page 64) by which glucal as a typical case can be converted into 2-deoxy glucose. Natural deoxy sugars such as 2-deoxy-D-ribose (2-ribodesose) occur in nucleic acids, and digitoxose, a 2,6-dideoxy hexose, and its 3-methyl derivative, cymarose, occur in the cardiac glycosides. These sugars are comparatively rare, however, and the 6- or ω-deoxy sugars, often called the methyl pentoses, are much more common.

---

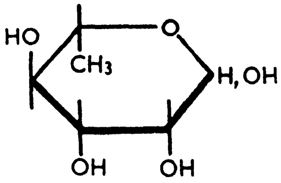
* *J.C.S.*, 1907, 1766, 1780; 1912, 2383.



L-Rhamnose
6-Deoxy-L-mannose
L-Mannomethylose

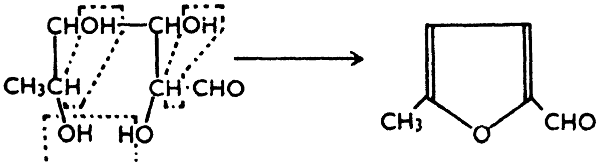


L-Fucose
6-Deoxy-L-galactose
L-Galactomethylose



L-Rhamnose

L-Rhamnose is found in many glycosides for example, quercitrin, and in such polysaccharides as slippery elm mucilage. L-Fucose occurs in gum tragacanth and is the main sugar building unit in the seaweed polysaccharide, fucoidin. The method of synthesising these 6-deoxy hexoses from the 6-toluenesulphonates by treatment with sodium iodide followed by reduction has been dealt with already (page 54). Of the general properties of these deoxy sugars it is only necessary to mention that they react in most respects like hexoses. One particular reaction of importance is the production of 5-methyl furfural on distillation with hydrochloric acid (12-13%); it will be remembered that pentoses on similar treatment (page 152) yield furfural. A method of estimating the so-called methyl pentoses is based on this reaction: the methyl furfural being precipitated with phloroglucinol or thiobarbituric acid. Since the amounts of methyl furfural produced vary slightly with different 6-deoxy hexoses it is necessary to use previously compiled tables to calculate the quantity originally present and difficulties arise when, as frequently happens, pentoses are present also, since furfural gives precipitates with the reagents.



5-Methyl furfural

An elegant method overcoming this difficulty has been introduced by Nicolet and Shinn* whereby periodic acid oxidises the 6-deoxy hexose to yield acetaldehyde from the terminal positions, the remainder of the molecule being converted to formic acid. The acetaldehyde is

---

* *J. Amer. Chem. Soc.*, 1941, 63, 1456.

$$CH_3(CHOH)_4CHO + 4\,HIO_4 = CH_3CHO + 4\,HCOOH + 4\,HIO_3$$

transferred by a current of carbon dioxide or nitrogen quantitatively into sodium bisulphite solution and estimated by titration with iodine after the addition of sodium bicarbonate. To prevent interference by hexoses or pentoses which if present would, with periodate, yield formaldehyde from the terminal $-CH_2OH$ groups, alanine is added to the reaction mixture to fix the formaldehyde as the formal derivative.

Ascorbic Acid

A. Szent Györgyi* isolated a substance from the adrenal cortex of the ox, and later from lemons and paprika, which he named hexuronic acid. With a molecular weight of 176 and a formula, $C_6H_8O_6$, the substance is acidic, very strongly reducing, optically active, and gives positive colour reactions with reagents used for detecting carbohydrates. A detailed study of its constitution was undertaken by Hirst† and some of the observations that led to the structural definition of this substance, which was recognised to be the antiscorbutic vitamin, Vitamin C, and eventually named ascorbic acid, are set out below.

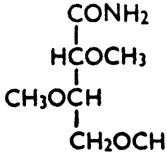
The powerful reducing action of ascorbic acid was illustrated by the fact that on titration with iodine two atoms of iodine were used up immediately and two molecules of hydrogen iodide were produced. The oxidised ascorbic acid (dehydroascorbic acid) so produced was capable of oxidising hydriodic acid again when the solution was evaporated, with the liberation of iodine and the regeneration of ascorbic acid. Alkaline hypoiodite solution oxidised ascorbic acid to give a molecule of oxalic acid whereas with acid permanganate an atom of oxygen was rapidly taken up, followed by a slower reaction when two atomic proportions were absorbed and carbon dioxide was evolved. The products of this reaction were methylated to give a trimethoxybutyric acid isolated as a crystalline amide, subsequently recognised to be trimethoxy L-threonamide.

The stereochemical configuration of part of the ascorbic acid molecule was established by oxidation with sodium hypochlorite followed by nitric acid. Methylation of the products, esterification, fractional distillation and amide formation gave L(+)-dimethoxy-succinamide in good yield, thus establishing the identity of the above trihydroxybutyric acid, and proving the relationship of ascorbic acid to the L-series of the sugars.

---

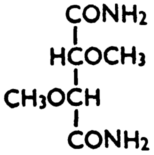
* *Biochem. J.*, 1928, **22**, 1387.

† Herbert, Hirst, Percival, Reynolds and Smith, *J.C.S.*, 1933, 1270.



$$\begin{array}{r}
\mathrm{CONH_2}\\
|\\
\mathrm{HCOCH_3}\\
|\\
\mathrm{CH_3OCH}\\
|\\
\mathrm{CH_2OCH_3}
\end{array}$$

Trimethoxy L-threonamide

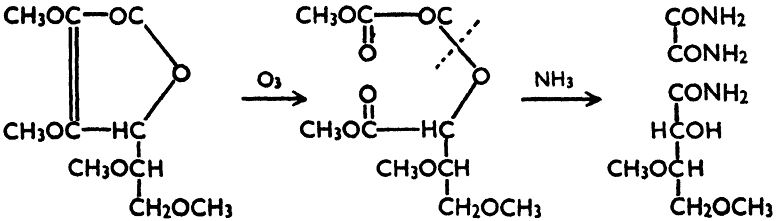


$$\begin{array}{r}
\mathrm{CONH_2}\\
|\\
\mathrm{HCOCH_3}\\
|\\
\mathrm{CH_3OCH}\\
|\\
\mathrm{CONH_2}
\end{array}$$

L(+)-Dimethoxy succinamide

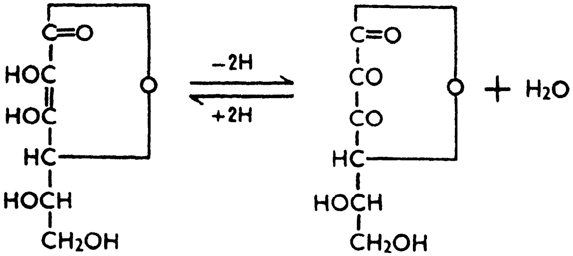
When ascorbic acid was distilled with hydrochloric acid as in a pentose estimation, furfural was obtained, showing that at least five of the six carbon atoms were present in an unbranched chain. With ferric chloride a colour typical of an enolic form was obtained, and it was found that the acidity of ascorbic acid was due to its enolic structure. Ascorbic acid behaved like a typical organic acid to indicators, it decomposed carbonates, appeared to form salts, and titrated sharply. It was found, however, that when ascorbic acid was oxidised with iodine, this acidic property disappeared, and the oxidised product could be titrated only slowly with sodium hydroxide as in the titration of a lactone. It was clear then that the oxidation with iodine, that is the removal of the two hydrogen atoms to give dehydroascorbic acid, had destroyed the seat of the acidity, which could not, therefore, have resided in a carboxyl group. This suggested that the acidity was due to an activated hydroxyl group, such as $-\mathrm{C(OH)=C(OH)}-$ which at once explained the extreme instability of ascorbic acid to alkaline reagents and its powerful reducing properties.

Strong support for this view came from a study of the action of ozone on fully methylated (tetramethyl) ascorbic acid, prepared by masking the reactive hydroxyl groups by methylation with diazomethane, followed by treatment with silver oxide and methyl iodide according to the Purdie method. Ozone was found to split the double bond to give a neutral " semioxalic ester ", which on treatment with ammonia gave oxamide and a dimethoxyhydroxybutyramide which, since it gave a positive Weerman reaction, was proved to be an α-hydroxy amide, and on methylation gave the trimethoxy-L-threonamide previously prepared. This experiment therefore de-



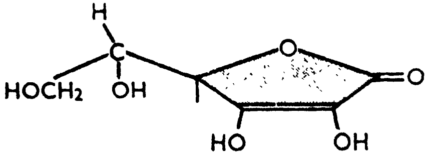
Tetramethyl ascorbic acid

cided that the ring in ascorbic acid must connect C_1 and C_4 and is therefore of the furane type. Ascorbic acid is accordingly given the dienol structure below and the so-called first oxidation product (dehydroascorbic acid) is derived from it by the loss of two atoms of hydrogen, which results in the disappearance of the double bond.



L-Ascorbic acid   Dehydroascorbic acid

X-Ray analysis gave full support to this formulation, the space model of which is exceedingly flat, for of the twelve carbon and oxygen atoms all but one lie in a plane and the remaining one (C_5) lies less than 1 Å above it.



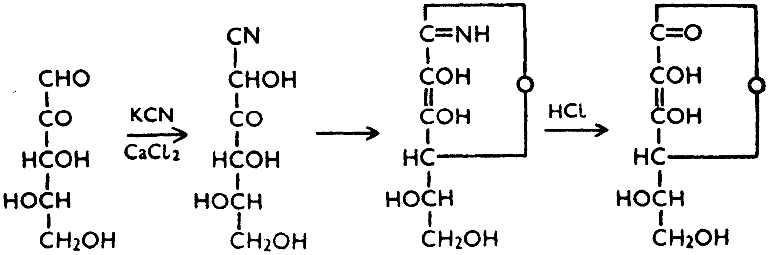
L-Ascorbic acid

Ascorbic acid was synthesised almost simultaneously by Reichstein* in Zürich, albeit starting with the false assumption that it possessed a furane carboxylic acid structure, and by Haworth, Hirst and their co-workers† in Birmingham, the starting point in each case being L-xylosone, which was treated with calcium chloride and potassium cyanide: the product resulting from the hydrolysis of the "imino" ascorbic acid which is formed as an intermediate was readily crystallised and proved to be identical with natural ascorbic acid. The L-xylosone was prepared from D-galactose as previously described (page 56). This method was by no means economical and more direct methods have since been devised. Before discussing them, however, it is worthy of note that biological tests showed that the synthetic L-ascorbic acid was as active as the natural material, but that D-ascorbic acid was quite inactive. Many analogues of ascorbic acid have been prepared since but none show the same antiscorbutic activity as ascorbic acid itself, the most active being 6-deoxy L-ascorbic acid which is about one third as active.*

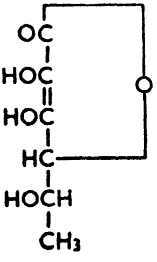
---

* Reichstein, Grüssner and Oppenauer, *Helv. Chim. Acta*, 1933, **16**, 1019.

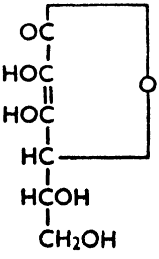
† Ault, Baird, Carrington, Haworth, Herbert, Hirst, Percival, Smith and Stacey, *J.C.S.*, 1933, 1419.



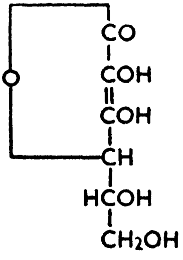
L-Xylosone "Imino" ascorbic acid L-Ascorbic acid



Activity (1/3)
6-Deoxy-L-ascorbic acid



(1/20)
D-Arabo-ascorbic acid

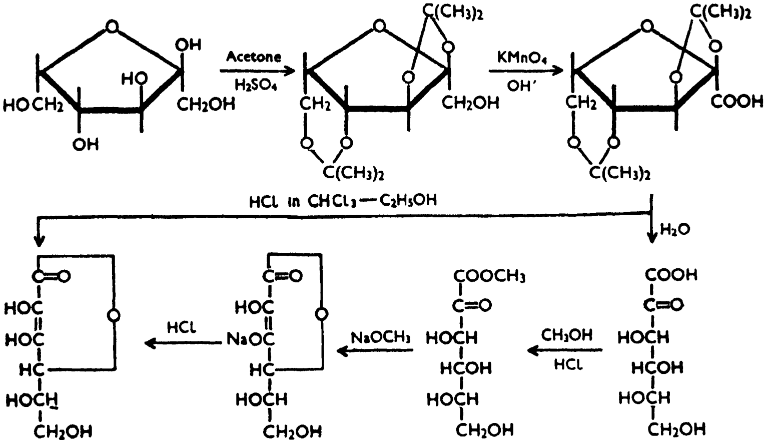


(0)
D-Xylo-ascorbic acid

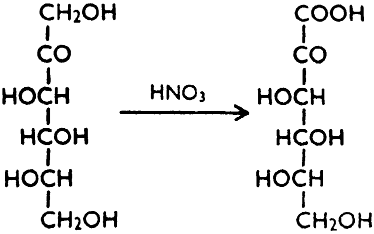
Ascorbic acid is made commercially from L-sorbose which is prepared from glucose *via* D-sorbitol as already outlined (page 193). L-Sorbose is condensed with acetone to give diacetone L-sorbofuranose which is oxidised with permanganate and the acetone groups removed by hydrolysis to give 2-keto-L-gulonic acid.

---

* See Smith, *Advances in Carbohydrate Chemistry*, 1946, **2**, 95.



It is also possible to prepare 2-keto-L-gulonic acid by the direct oxidation of L-sorbose with nitric acid under controlled conditions.



L-Sorbose 2-Keto-L-gulonic acid

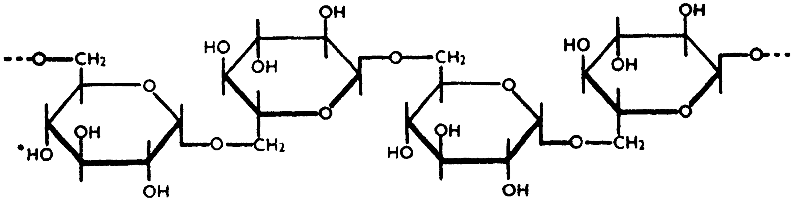
CHAPTER XI

SOME LESS FAMILIAR POLYSACCHARIDES

Polyglucosans

Dextrans

SLIMY polysaccharides known as dextrans were frequently encountered in the middle of the last century in such processes as the manufacture of sugar from beet, and in the wine industry, and proved a serious handicap, retarding both filtration and crystallisation. These substances are produced from sucrose by the agency of *Leuconostoc* organisms,* although their fundamental unit is glucose. *Leuconostoc dextranicum* produces a dextran, which after complete methylation and hydrolysis gives 2,3,4-trimethyl glucose (90% yield), together with some unidentified dimethyl glucoses.† The essential feature of this dextran is a chain of D-glucopyranose units joined by α-linkages through the 1,6-positions. The question of whether the polysaccharide is a network of such chains with the dimethyl glucoses at the branching points or a long chain considered to contain at least 200 glucopyranose units is not finally decided.



Chain of α-Glucopyranose Units in Dextran

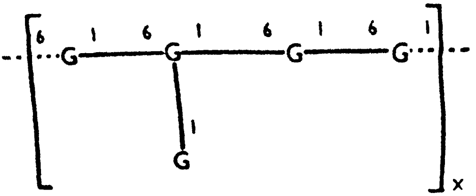
The organism *Leuconostoc mesenteroides* elaborates a dextran which appears to be of somewhat different configuration although in this case also a 1,6-link is present. The fully methylated dextran on hydrolysis with methanolic hydrogen chloride gave 2,3,4,6-tetramethyl methylglucoside (1 part), 2,3,4-trimethyl methylglucoside (3 parts) and 2,3-dimethyl methylglucoside (1 part).‡ These results obviously indicate a branched chain structure such as that given in symbolic form below, although other variants are possible.

---

* See Evans and Hibbert, *Advances in Carbohydrate Chemistry*, 1946, **2**, 203, Academic Press, New York.

† Peat, Schlüchterer and Stacey, *J.C.S.*, 1939, 581.

‡ Fairhead, Buckland, Brauns and Hibbert, *Can. J. Research*, 1937, **B.15**, 486; Levi, Hawkins and Hibbert, *J. Amer. Chem. Soc.*, 1942, **64**, 1959.



Branched Chain Structure of Repeating Unit of
Leuconostoc mesenteroides Dextran
(G = glucose)

Another strain of *Leuconostoc mesenteroides* (*Betacoccus arabinosaceus* Orla-Jensen) metabolised a dextran from which on methylation and hydrolysis 2,3,4-trimethyl- and 2,3,4,6-tetramethyl-glucose were isolated in the ratio of 5 : 1. The molecular weight reported was relatively low being 11,700 from viscosity measurements and only 2,600 from a measurement of the sedimentation equilibrium.* The product may nevertheless be a branched chain polysaccharide. It will be noted that the essential feature of these bacterial dextrans is the 1,6-linkage. By the hydrolysis of a *leuconostoc* dextran and acetylation, a crystalline octaacetyl 6-[α-D-glucopyranosido]-β-glucose has been prepared.†

Laminarin

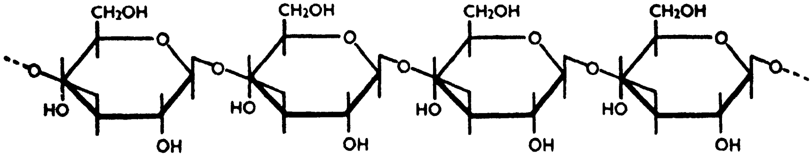
Laminarin is another polysaccharide constructed entirely of D-glucose residues. This substance may be isolated from certain brown seaweeds, especially of the *Laminaria spp.*, by extraction either with water, or, more readily with dilute acids. When fronds of *Laminaria cloustoni* are steeped in dilute hydrochloric acid, a snowy white powder is deposited, which, after washing with water may be redissolved in hot water and is reprecipitated on cooling.‡ Dry fronds of seaweeds of the *Laminaria spp.* collected in autumn may contain over 30% of laminarin.

Hydrolysis with dilute mineral acids gave D-glucose in 99% yield as estimated polarimetrically as the only recognisable product. On methylation and hydrolysis of the polysaccharide 2,4,6-trimethyl glucose resulted,§ and from the specific rotation of laminarin

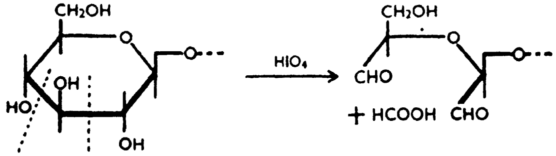
---

* Hassid and H. A. Barker, *J. Biol. Chem.*, 1940, **134**, 163.
† Georges, Miller and Wolfrom, *J. Amer. Chem. Soc.*, 1947, **69**, 473.
‡ Barry, *Sci. Proc. Roy. Dublin Soc.*, 1938, **21**, 615.
§ Barry, *Sci. Proc. Roy. Dublin Soc.*, 1939, **22**, 59.

($[\alpha]_D$ – 13°) it is clear that β-linkages are involved. The disaccharide laminaribiose has been mentioned already (Chapter V) and is obtained by the hydrolysis of laminarin either with oxalic acid or with an enzyme from snail juice. The arrangement as chains of β-glucose units linked through positions 1 and 3 was confirmed by experiments with periodic acid.* The 1,3-link precludes the possibility of the presence of adjacent hydroxyl groups in the glucose units. Consequently periodic acid would not be expected to attack laminarin, although cellulose and starch with the 1,4-links and adjacent hydroxyl groups on C_2 and C_3 consume approximately one molecule of periodic acid for each $C_6H_{10}O_5$ unit.† In practice Barry‡ found that the consumption of periodic acid was small, and that this was accounted for by oxidation at the terminal position of the chain.



Chain of β-Glucopyranose Units in Laminarin



Oxidation of Terminal Glucopyranose Unit in Laminarin

By oxidation with bromine and titration of the dicarboxylic acid which resulted, it was estimated that the number of β-1,3-linked glucose units in a terminated chain was sixteen, although the last word has not yet been said on the structure of this polysaccharide.

Yeast Glucan

It is of interest to note that yeast contains, apparently associated with the cell walls, an insoluble polysaccharide usually called yeast glucan, with the same fundamental structure as laminarin, since the only product obtained on hydrolysing the methylated polysaccharide was 2,4,6-trimethyl glucose,§ and the links are β- as deduced by enzymic hydrolysis. Oxidation with periodic acid shows* one end group for every twenty-eight glucopyranose units.

---

* Barry, Dillon and McGettrick, *J.C.S.*, 1942, 183.

† Jackson and Hudson, *J. Amer. Chem. Soc.*, 1937, **59**, 2049; 1938, **60**, 989.

‡ *J.C.S.*, 1942, 578.

§ Zechmeister and Tóth, *Biochem. Z.*, 1934, **270**, 309; Hassid, Joslyn and McCready, *J. Amer. Chem. Soc.*, 1941, **63**, 295.

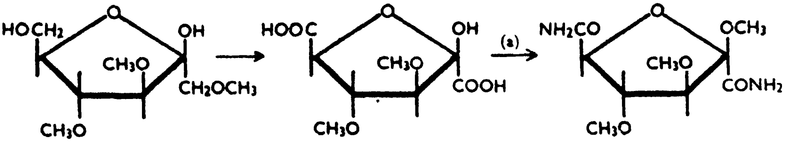
It is clear from the brief accounts of the dextrans, laminarin and yeast glucan that the mode of linking of glucose units in polyglucoses is not by any means invariably by the familiar 1,4-links found in starch and cellulose.

Polyfructosans

Levan

Solutions of sucrose and raffinose are attacked by organisms such as *B. mesentericus*, *B. vulgatus* and *B. subtilis* with the production of a fructosan† which appears as a gummy product in the solution, the production of this substance being from time to time a source of difficulty during sugar manufacture.

Gum levan, or levan as it is usually called, gives a high yield (99%) of crystalline fructose on hydrolysis, and may be readily converted into a triacetate and a trimethyl derivative.† Hibbert and his co-workers showed that on hydrolysing methylated levan a crystalline trimethyl fructose was obtained. This substance failed to give an osazone, indicating substitution on C_1. Complete methylation and hydrolysis of the tetramethyl methylfructoside gave, not crystalline tetramethyl fructopyranose but tetramethyl fructofuranose ($[\alpha]_D + 31°$; page 93), so that the hydroxyl group on C_5 was unsubstituted. The choice thus lay between 1,3,4-trimethyl- (I) and 1,3,6-trimethyl-fructose. This latter possibility was negatived by the isolation, after oxidation with nitric acid, of a dimethyl 2-keto-saccharic acid (II), which was crystallised in the form of a diamide (III) after simultaneous esterification and glycoside formation followed by treatment with ammonia. The trimethyl fructose from methylated levan was therefore 1,3,4-trimethyl fructose. This



I II III

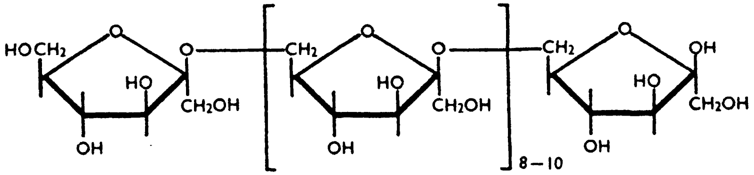
(*a*) = $CH_3OH - HCl$ followed by NH_3

---

* Barry and Dillon, *Proc. Roy. Irish Acad.*, 1943, **490**, 177.

† Hibbert, Tipson and Brauns, *Can. J. Research*, 1931, **4**, 221.

fact alone was obviously insufficient to settle the constitution of levan, since pyranose rings with 2,5-links and furanose rings with 2,6-links are alternative possibilities. The ease of hydrolysis of levan, however, is strong evidence for the presence of fructofuranose residues. The mean rate of hydrolysis in normal oxalic acid solution at 65° was found to lie between the values for inulin and sucrose.* Independent support for this view was provided by an end group assay carried out in the Birmingham laboratories† for the hydrolysis of a methylated levan prepared from *B. mesentericus* gave between 10% and 11% of tetramethyl fructofuranose characterised as the crystalline 2,3,4,6-tetramethyl 2-keto-gluconamide. The chain of levan is therefore judged to be constructed of between ten and twelve fructofuranose units joined through the reducing group by β-linkages (chosen on account of the specific rotation) to the primary alcoholic residue on C_6. Although the fructofuranose residue on the right hand side of the formula is represented as containing a reducing group, there is a degree of uncertainty on this score and the above formula, as for many polysaccharides, is only to be considered as a first approximation.



Chain of Fructofuranose Units in Levan

Whilst, as in inulin (page 118) the reducing group of one fructofuranose unit is joined by a β-link to the primary alcoholic group in an adjoining unit, there is the highly important difference that it is the group on C_6 and not that on C_1 which is involved in levan.

A levan which appears to be identical with that synthesised by the aid of enzymes secreted by bacteria, is found in rough stalked meadow grass (*Poa trivialis*) and also in barley leaves‡ and is presumably an important article of diet for farm animals. Hydrolysis of the methylated grass levan gave the crystalline 1,3,4-trimethyl fructose discussed above.§

---

* Hibbert and Percival, *J. Amer. Chem. Soc.*, 1930, **52**, 3995.
† Challinor, Haworth and Hirst, *J.C.S.*, 1934, 676.
‡ Haworth, Hirst and Lyne, *Biochem. J.*, 1937, **31**, 786.
§ Challinor, Haworth and Hirst, *J.C.S.*, 1934, 1560.

Other Polyfructosans

A very exhaustive study of polyfructosans has been carried out by Schlubach and his School.*

The polyfructosans are classified in two main groups, those which possess 1,2-links like inulin, sinistrin (from the sea onion), graminin (from rye), asparagosin (from asparagus), and those with 1,6-links such as levan, phlein (timothy grass) and secalin (unripe rye). The isolation of dimethyl fructoses from the hydrolysis of the methylated polysaccharides is taken as evidence of branched chain structures.

One polyfructosan, *irisin*, from roots of species of Iris gives on methylation and hydrolysis only tetramethyl fructofuranose and a dimethyl fructofuranose in equal proportions; from this it is concluded† that fructofuranose units are arranged in the form of a ring with other fructofuranose units attached by glycosidic union; it is these latter which give the tetramethyl fructofuranose, and the ring residues, which are triply linked, give rise to the dimethyl fructose.

Another interesting polyfructosan is *triticin* isolated from couch grass.‡ This compound is relatively stable and the methylated polysaccharide gives tetra-, tri- and di-methyl fructoses in the proportions of 3 :1 :3 on hydrolysis, and triticin is thought to be made up of a closed ring of fructofuranose residues with a multiple of seven units (either 14 or 21) in the ring, the molecular weight corresponding to between 16 and 17·5 fructose residues. It is to be presumed that the fructose units appearing ultimately as tetramethylfructofuranose are attached to the loop and radiate from it.

Schlubach believes that in general the polyfructosans are not straight chain compounds, but that the units are joined together in the form of closed loops. This is a very difficult matter to decide. Although inulin for example appears to have a chain structure, the polysaccharide is so easily hydrolysed that the possibility remains that the substance may occur in the plant in the form of a closed ring.

Polygalactans

Polysaccharides containing galactose as the only structural unit are not so common as the polyglucosans and polyfructosans although many complex polysaccharides such as the plant gums contain galactose in association with other sugars. Structural studies are therefore not so far advanced as in the glucose series.

---

* Schlubach and Sinh, *Annalen*, 1940, **544**, 101; see E. J. McDonald. *Advances in Carbohydrate Chemistry*, 1946, **2**, 256, Academic Press, New York,

† Schlubach, Knoop and Liu, *Annalen*, 1933, **504**, 30.

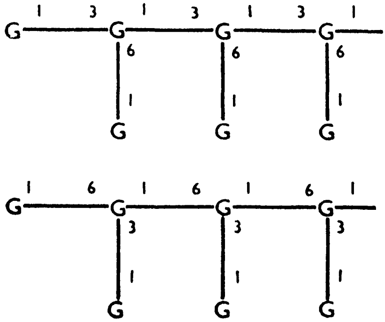
‡ Schlubach and Peitzner, *Annalen*, 1937, **530**, 120.

Snail Galactogen

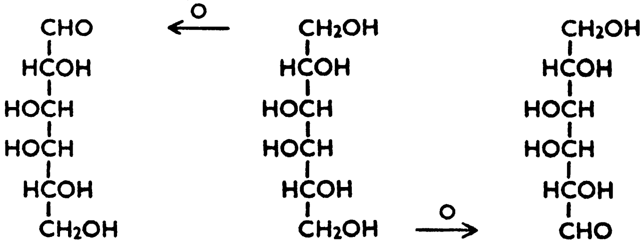
The edible snail *Helix pomatia* secretes a galactogen which appears to be a reserve polysaccharide like glycogen, although glycogen is also present in the snail and the galactogen is only utilised by the fasting snail if its supplies of glycogen are seriously depleted.

The methylated galactogen in question gives on hydrolysis equal parts of 2,3,4,6-tetramethyl galactose and 2,4-dimethyl galactose.* It is concluded that the polysaccharide consists of a backbone of galactopyranose units linked through either C_1 or C_3 with the pyranose end group on C_6, or joined through C_1 and C_6 with the addenda on C_3, there being seven residues in a structural unit. There is a special point of interest in that one of every seven galactopyranose residues is found to be an L-galactose unit which is one of the side chains. The occurrence of L-galactose alongside D-galactose is not infrequent; for it is found also in the polysaccharide isolated from agar (page 217), and it may be connected with the fact that in the formation of galactose by the oxidation of the hexahydric alcohol, dulcitol, if oxidation occurs on C_1 D-galactose, and if on C_6 L-galactose results.

Alternative possible formations of Snail Galactogen



(G = galactose)



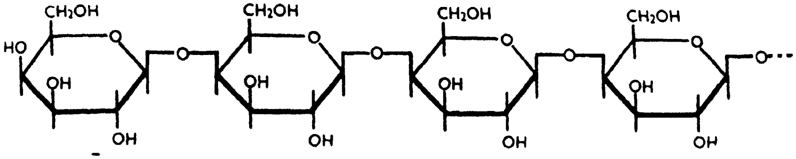
D-Galactose Dulcitol L-Galactose

---

* Baldwin and Bell, *J.C.S.*, 1938, 1461; 1941, 125.

Pectin Galactan

A galactan is associated with pectic acid (page 156) in fruit pectin. This substance on methylation and hydrolysis yields 2,3,6-trimethyl- and 2,3,4,6-tetramethyl-D-galactose. From the low value of the specific rotation of this substance the presence of β-links is inferred and the main features of its structure* are represented by a chain of β-D-galactopryanose units linked through C_1 and C_4.

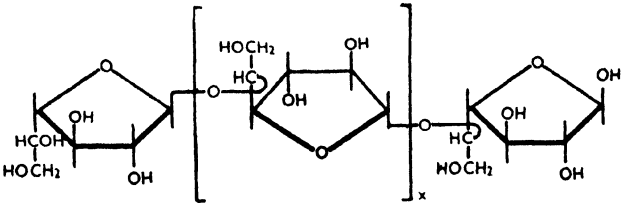


Chain of β-Galactopyranose Units in Pectin Galactan

From the proportion of tetramethyl galactopyranose isolated it is calculated that a repeating unit of about 120 galactopyranose units is the main feature of this galactan molecule.

Galactocarolose

To illustrate the diversity of the galactose group of polysaccharides, the substance galactocarolose may be mentioned. This substance is synthesised by the action of a mould, *Penicillum Charlesii*, G. Smith, on a substrate containing galactose. On hydrolysis of the methylated polysaccharide 2,3,6-trimethyl galactose and 2,3,5,6-tetramethyl D-galactofuranose were obtained, the latter unit presumably existing as an end group. Since galactocarolose, unlike the pectin galactan just described which is very stable, is very easily hydrolysed by dilute acids, and since the terminal unit must be furanosido, it is concluded that the main structure of this galactan is represented by



Galactocarolose

a chain of β-galactopyranose units linked through C_1 and C_6.†

---

* Hirst, J. K. N. Jones and Walder, *J.C.S.*, 1947, 1225.

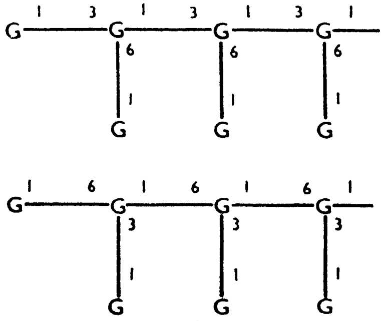
† Haworth, Raistrick and Stacey, *Biochem. J.*, 1937, **31**, 640.

Snail Galactogen

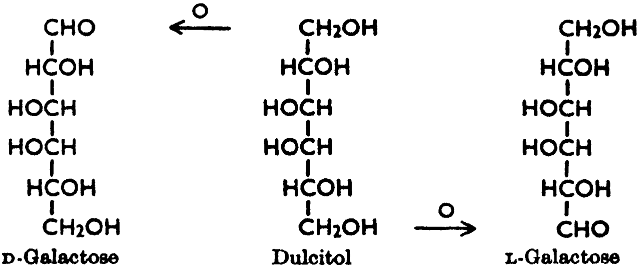
The edible snail *Helix pomatia* secretes a galactogen which appears to be a reserve polysaccharide like glycogen, although glycogen is also present in the snail and the galactogen is only utilised by the fasting snail if its supplies of glycogen are seriously depleted.

The methylated galactogen in question gives on hydrolysis equal parts of 2,3,4,6-tetramethyl galactose and 2,4-dimethyl galactose.* It is concluded that the polysaccharide consists of a backbone of galactopyranose units linked through either C_1 or C_3 with the pyranose end group on C_6, or joined through C_1 and C_6 with the addenda on C_3, there being seven residues in a structural unit. There is a special point of interest in that one of every seven galactopyranose residues is found to be an L-galactose unit which is one of the side chains. The occurrence of L-galactose alongside D-galactose is not infrequent; for it is found also in the polysaccharide isolated from agar (page 217), and it may be connected with the fact that in the formation of galactose by the oxidation of the hexahydric alcohol, dulcitol, if oxidation occurs on C_1 D-galactose, and if on C_6 L-galactose results.

Alternative possible formations of Snail Galactogen



(G = galactose)

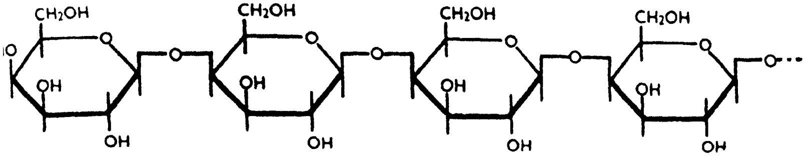


---

* Baldwin and Bell, *J.C.S.*, 1938, 1461; 1941, 125.

Pectin Galactan

A galactan is associated with pectic acid (page 156) in fruit pectin. This substance on methylation and hydrolysis yields 2,3,6-trimethyl- and 2,3,4,6-tetramethyl-D-galactose. From the low value of the specific rotation of this substance the presence of β-links is inferred and the main features of its structure* are represented by a chain of β-D-galactopryanose units linked through C_1 and C_4.

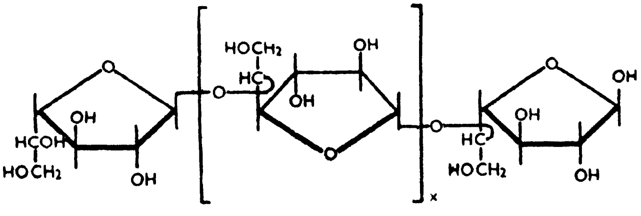


Chain of β-Galactopyranose Units in Pectin Galactan

From the proportion of tetramethyl galactopyranose isolated it is calculated that a repeating unit of about 120 galactopyranose units is the main feature of this galactan molecule.

Galactocarolose

To illustrate the diversity of the galactose group of polysaccharides, the substance galactocarolose may be mentioned. This substance is synthesised by the action of a mould, *Penicillum Charlesii*, G. Smith, on a substrate containing galactose. On hydrolysis of the methylated polysaccharide 2,3,6-trimethyl galactose and 2,3,5,6-tetramethyl D-galactofuranose were obtained, the latter unit presumably existing as an end group. Since galactocarolose, unlike the pectin galactan just described which is very stable, is very easily hydrolysed by dilute acids, and since the terminal unit must be furanosido, it is concluded that the main structure of this galactan is represented by



Galactocarolose

a chain of β-galactopyranose units linked through C_1 and C_5.†

---

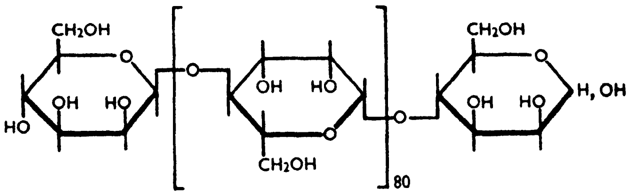
* Hirst, J. K. N. Jones and Walder, *J.C.S.*, 1947, 1225.

† Haworth, Raistrick and Stacey, *Biochem. J.*, 1937, **31**, 640.

Polymannosans or Mannans

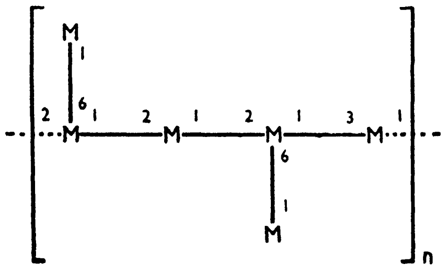
D-Mannose is found combined as the structural unit in the polysaccharides called mannans, in fact the sugar mannose is usually obtained from the polysaccharides contained in the seeds of the ivory nut palm, the so-called vegetable ivory. Two mannans "A" and " B " appear to be present in the ivory nut, both of which have the same kind of link, the mannose units being joined through C_1 and C_4 by β-links as in cellulose.*

The evidence for this lies in the isolation of 2,3,6-trimethyl mannose as the chief hydrolysis product of the methylated mannans. The so-called Mannan "A" gives tetramethyl D-mannopyranose on hydrolysis in such quantity that a chain length of about 80 units is envisaged.



Yeast contains a mannan which unlike the ivory nut mannans has a branched chain structure. Hydrolysis of the methylated yeast gum gave approximately equimolecular proportions of tetramethyl D-mannopyranose, 3,4-dimethyl mannose, and a mixture of trimethyl mannoses. The latter was resolved into approximately equal amounts of 3,4,6- and 2,4,6- together with less than 10% of 2,3,4-trimethyl D-mannose. The different links present are clearly 1,2; 1,3; and 1,6, and a branched chain structure must be involved with the dimethyl mannose at the branching points.†

Yeast Mannan, One representation of the Repeating Unit



---

* Klages, *Annalen*, 1934, **509**, 159.

† Haworth, Hirst and Isherwood, *J.C.S.*, 1937, 784; Haworth, Heath and Peat, *J.C.S.*, 1941, 833.

Several types of structure are possible, one *repeating unit which* agrees with the facts is set out at the foot of page 214.

Mannose is also found, associated with other *sugars, in other com*plex polysaccharides such as ovomucoid, a complex carbohydrate found in white of egg, and in many plant gums.

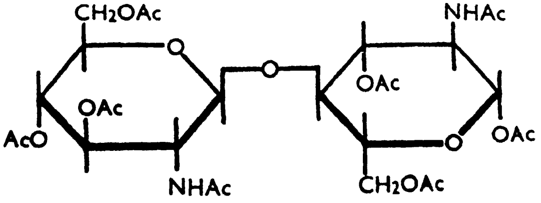
Polysaccharides containing Nitrogen

Chitin

Lobster and crab shells contain an amino polysaccharide, chitin, which may be isolated as a leathery substance after dissolution of the calcium carbonate with mineral acids and removal of the proteins. Furthermore chitin is found as a structural carbohydrate in the arthropods, molluscs, brachyopods, bryozoa and similar species, and also in fungi, although it is not known whether fungal chitin and animal chitin are identical in all respects.

The polysaccharide can be isolated as a colourless amorphous powder which is insoluble in water, dilute mineral acids and alkalis, and in these respects resembles cellulose; it is, however, insoluble in Schweizer's reagent. Dissolution takes place in concentrated mineral acids, and chitin, apparently unchanged, is precipitated on dilution, although hydrolysis takes place on long standing ($[\alpha]_D$ − 15° ⟶ + 56°) and glucosamine and an equivalent quantity of acetic acid are released. N-Acetyl glucosamine has been isolated by cautious hydrolysis with acid, but better results are achieved using *chitinase*, an enzyme preparation found in the intestinal tract of snails, a yield of N-acetyl glucosamine amounting to 85% having been recorded.

Chitin has been subjected to acetolysis,* an octaacetyl chitobiose which is deemed to be analogous to octaacetyl cellobiose being obtained. Six of the eight acetyl groups were easily hydrolysed by



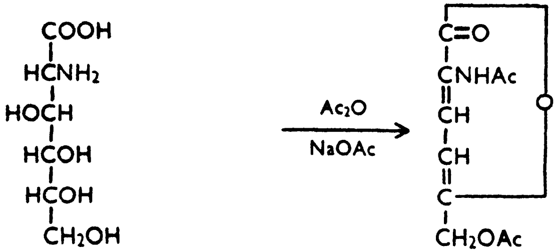
Octaacetyl chitobiose

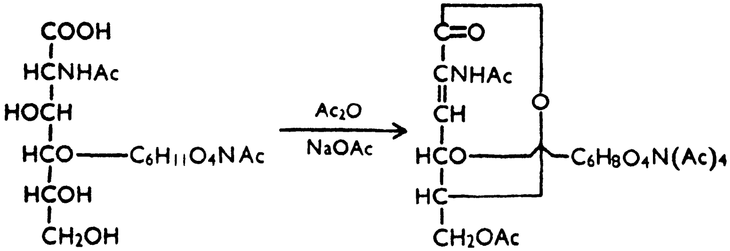
the action of sodium hydroxide; this would agree with the presence of two N-acetyl residues, which are much more stable to alkali than

---

* Bergmann, Zervas and Silberkweit, *Ber.*, 1931, **64**, 2436.

O-acetyl groups. An estimation of the reducing power with sodium hypoiodite, after deacetylation, indicated the presence of one reducing group and the corresponding chitobionic acid reacted with acetic anhydride and sodium acetate in a way which was in harmony with the presence of a linkage to C_4. Thus glucosaminic acid on heating with acetic anhydride and sodium acetate gives rise to an unsaturated lactone containing two double bonds, between C_2 and C_3, and





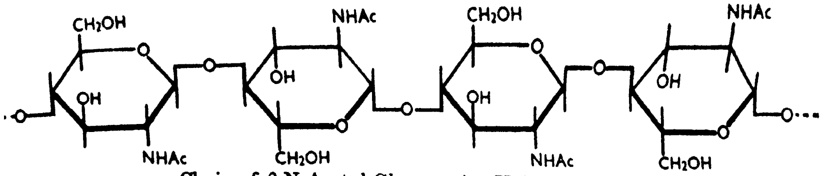
C_4 and C_5. On similar treatment chitobionic acid gave an unsaturated lactone with only a single double bond which proved to be between C_2 and C_3 since ozonisation yielded acetyl oxamic acid HOOC.CONHAc. It is concluded from this therefore that the hydroxyl group on C_4 is substituted.

By the partial hydrolysis of chitin with concentrated hydrochloric acid, followed by acetylation, Zechmeister and Tóth* isolated a chitotriose hendecaacetate so that chitin behaves in the same way as cellulose in this respect also, and as a result of the study of the X-ray diagram given by chitin, the suggestion that cellulose and chitin are structurally similar has been made.†

---

* *Ber.*, 1931, **64**, 2028; 1932, **65**, 161.

† Meyer and Mark, *Ber.*, 1928, **61**, 1936.

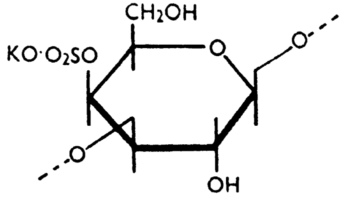


Chain of β-N-Acetyl Glucosamine Units in Chitin

Polysaccharides containing Sulphur

Seaweed polysaccharides frequently contain sulphur in the form of an ethereal sulphate group $>CHOSO_2O^-$, and these compounds occur as salts in the algae. The physical properties of these polysaccharides vary therefore according as the cations are monovalent or divalent. Thus the carragheen polysaccharide ethereal sulphates of *Chondrus crispus* and *Gigartina stellata* (the so-called carrageenin) contain about 8% of sulphur as sulphate, and are a mixture of salts chiefly of sodium, potassium, magnesium, and calcium. When all the monovalent ions are replaced by calcium the polysaccharide forms a gel, whereas the sodium and potassium salts give viscous solutions in water. This difference is due presumably to cross linking between chains by the divalent calcium ions, in the same way as mentioned already for alginic acid.

The complete structure of these polysaccharides has not yet been worked out, but it is held that the principal building unit is D-galactopyranose linked through C_1 and C_3 and carrying an ethereal sulphate residue on C_4.*



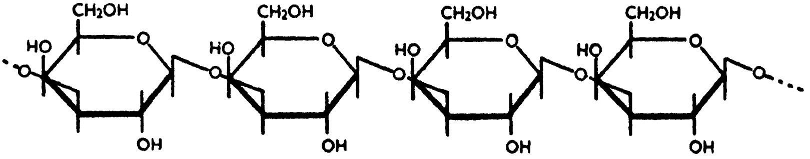
Another important polysaccharide of the red seaweeds is agar. This substance is elaborated by seaweeds of the *Gelidium spp.* and is obtained mostly from Japan; agar bearing seaweeds such as *Gracilaria confervoides* are, however, indigenous to the coasts of Britain.

The isolation of 2,4,6-trimethyl D-galactose as the main hydrolysis product of methylated agar† showed for the first time in a polysaccharide the presence of D-galactopyranose units linked through the 1,3 positions.

---

* Buchanan, Percival and Percival, *J.C.S.*, 1943, 51; Dewar and Percival, *J.C.S.*, 1947, 1622.

† Percival and Somerville, *J.C.S.*, 1937, 1615.



Chain of Galactopyranose Units in Agar

Later L-galactose was found in the form of its 3,6-anhydride.* The amount of ethereal sulphate in agar is very much smaller than in the carragheen polysaccharides, the sulphur content being less than 0·5%,† but the isolation of derivatives of 3,6-anhydro L-galactose suggests the possibility that at some stage, those units which appear eventually as anhydro L-galactose residues, might have been ethereal sulphates‡ for the hydrolysis of simple sugar sulphates with alkali has been shown to yield anhydro sugars (page 68).

In the brown seaweeds another polysaccharide ethereal sulphate is found, called fucoidin. In this instance the main sugar building stone is not D-galactose but L-fucose,§ and the sulphate content is very high (S, 12%).

Polysaccharides containing Nitrogen and Sulphur

Complex polysaccharides which contain both nitrogen (in amino hexose units) and sulphur (as sulphates) are of great importance in the animal world. Outstanding examples are the mucopolysaccharides such as chondroitin sulphate, obtained from such sources as the nasal septa, tendons, trachea and aorta, which yield on hydrolysis N-acetyl chondrosamine (galactosamine), sulphuric acid, and glucuronic acid; mucoitin sulphate from the gastric mucosa which gives sulphuric acid, N-acetyl glucosamine and a hexuronic acid; and hyaluronic acid sulphate from the cornea of the eye and the umbilical cord, which contains N-acetyl glucosamine, glucuronic acid and sulphuric acid. Heparin is another substance of this type.

The complex character of such carbohydrates and their peculiar physical properties has made progress difficult in determining how the various components are linked together. A structure of the type given below has been suggested for chondroitin sulphate* on the grounds that 2,3,4-trimethyl β-methyl-D-glucuronoside and N-acetyl 3,4,6-trimethyl methylchondrosaminide† were isolated from a methylated degraded sulphate free chondroitin.

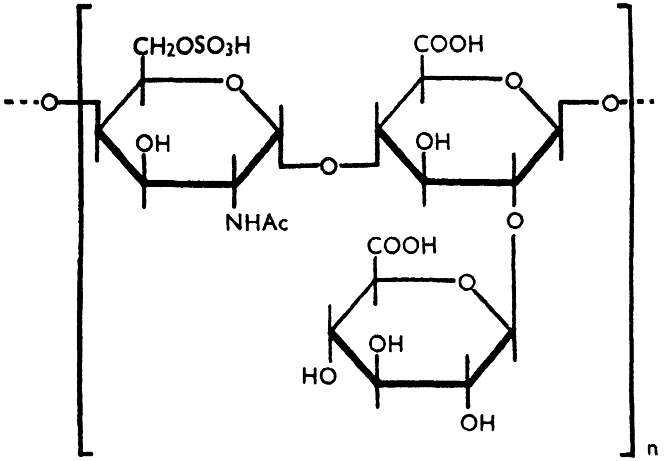
---

* Hands and Peat, *Chem. and Ind.*, 1938, **57**, 937; Forbes and Percival, *J.C.S.*, 1939, 1844.

† Barry and Dillon, *Chem. and Ind.*, 1944, **63**, 167; Percival, *Nature*, 1944, **154**, 673.

‡ Jones and Peat, *J.C.S.*, 1942, 225; Percival and Thomson, *ibid.*, 1942, 750.

§ Lunde, Heen and Öy, *Z. physiol. Chem.*, 1937, **247**, 189.



Another formulation has been suggested based on the results of periodate oxidation‡ in which chondroitin sulphate is depicted as a chain of about sixty residues of N-acetyl chondrosamine and glucuronic acid units linked through 1,3- and not 1,4-linkages.

While it will undoubtedly take some little time to unravel the complete constitution of such compounds as chondroitin and mucoitin sulphate, the general progress in polysaccharide chemistry in the past decade encourages the belief that substantial progress in that direction will not be long delayed.

---

[*] Haworth, *J.C.S.*, 1947, 582.

[†] Bray, Gregory and Stacey, *Biochem. J.*, 1944, **38**, 142

[‡] Meyer, Odier and Siegrist, *Helv. Chim. Acta*, 1948, **31**, 1400.

AUTHOR INDEX

A

Aiyar, 105
Alberda van Eckenstein, 16, 26
Appel, 170
Anderson, C. G., 55
Anderson, E., 164
Anger, 182
Armstrong, E. F., 27, 43, 94
Astbury, 136
Ault, 56, 154, 203
Auerbach, 168
Averill, 125
Avery, 38

B

Bacon, 118
Baird, 56, 116, 203
Baker, W., 175
Baldwin, E., 118, 212
Baldwin, R., 135
Barker, C. C., 51, 52, 143
Barker, H. A., 99, 138, 207
Barker, S. A., 146
Barry, 90, 207, 208, 209, 218
Bawn, 139, 141
Bear, 148
Beavan, 156
Beevers, 94
Bell, D. J., 118, 168, 212
Bell, F. O., 136
Bergmann, 62, 63, 111, 182, 215
Bernfeld, 116, 133, 135, 144
Binkley, 98
Bodycote, 78
Böeseken, 29, 30
Boezaardt, 198
Boissonnas, 112
Bonner, 82
Boppel, 143
Bott, 62
Bourne, 146
Bourquelot, 98, 99
Boutron-Charlard, 180
Brauns, 206, 209
Bray, 219
Bredereck, 87
Bridel, 98
Brigl, 58, 69
Brown, 129, 141, 169
Buchanan, 217
Buckland, 206
Butlerow, 23
Bywater, 148, 149

C

Campbell, 180
Carr, 193, 194
Carrington, 56, 203
Carter, 112, 120, 147
Chaikoff, 118
Challinor, 210
Chanda, 149
Charlton, 55
Cochrane, 94
Coleman, 87, 97
Colin, 170
Colley, 6
Colowick, 136, 138
Consden, 168
Cori, C. F., 136, 148
Cori, G. T., 136, 137, 148
Cox, 28, 75
Croft Hill, 99
Cutler, 75

D

Dale, 53
Delbrück, 61, 97
Denham, 105
Dewar, 54, 217
Dillon, 208, 209, 218
Dore, 99, 109, 116
Doudoroff, 99, 138
Drew, 34, 37, 38, 120
Dubrunfaut, 26
Duff, 68
Dunstan, 129

E

Edwards, 134, 135
Evans, 105
Everest, 176

F

Fairhead, 206
Fay, 15
Fischer, E., 2, 3, 6, 8-11, 14, 15, 19, 21, 23, 25, 26, 32, 37, 54, 60-63, 68, 74, 78, 83, 97, 172, 182
Fischer, H. O. L., 15
Fleury, 5

Flood, 168
Flynn, 75
Folkers, 75
Forbes, 218
Franchimont, 26
French, 134, 135
Freudenberg, K., 50, 55, 90, 95, 106, 107, 108, 134, 143
Freudenberg, W., 194
Friese, 106
Fuld, 147
Fyfe, 38

G

Gadamer, 183
Georges, 108, 207
Gerecs, 66, 88, 97
Gilbert, 95, 97
Gill, 155, 164
Gladding, 68
Goebel, 155, 162
Goepp, 194
Goodyear, 34, 38, 43, 55
Gordon, 168
Gralén, 125, 158
Granichstädten, 129
Gregory, 219
Griffith, 70
Gross, 16
Grüner, 58
Grüssner, 203
Guègen, 170
Gürtler, 112
Gulland, 188

H

Halsall, 118, 129, 141, 144
Hampton, 121
Hands, 218
Hanes, 133, 134, 136
Hann, 6, 54, 66, 96
Harris, 129
Hartzog, 160
Haskins, 54, 96
Hassid, 99, 116, 117, 133, 137, 138, 207, 208
Hawkins, E. G. E., 142
Hawkins, W. L., 206
Haworth, 32-35, 37, 38, 40, 43, 53, 55-58, 60, 62, 68, 75, 78, 80, 83-86, 88, 92, 93, 98, 100, 107, 109-111, 114, 116-122, 125, 126, 137, 144, 146, 148, 149, 154, 180, 203, 210, 213, 214, 219
Heath, 137, 214
Heddle, 52, 129
Heen, 218
Heidelberger, 155, 163
Helferich, 52, 87, 96, 97, 170
Hendricks, 50
Herbert, 56, 201, 203
Hess, 106, 125, 127, 133
Heuser, 104, 105
Hibbert, 107, 115, 119, 206, 209, 210,
Hickinbottom, 98
Hiller, 47
Hirst, 23, 33-35, 38, 40, 51-53, 56, 60, 62, 63, 78, 83, 86, 88, 93, 100, 105-107, 114, 116, 118, 120, 121, 125, 129, 139, 140, 141, 143, 144, 149, 150, 154-156, 158, 159, 164, 167-169, 201, 203
Hixon, 168
Hobby, 163
Hockett, 5, 12, 52, 192
Hogg, 38
Holly, 75
Hotchkiss, 155
Hough, 23, 169
Howard, 187
Hudson, 6, 9, 28, 36, 40, 43, 52-54, 66, 67, 69, 88, 89, 91, 94, 95, 96, 101, 193, 194, 208
Hynd, 75

I

Irvine, 32-34, 38, 67, 75, 92, 98, 105
Isbell, 43
Isherwood, 118, 139, 214

J

Jackson, E. L., 43, 67, 91, 208
Jackson, J., 68, 166
Jacobs, 21
James, 76
Jayme, 149
Jeanloz, 118
Jeffrey, 75
Joslyn, 208
Jones, D. I., 34, 35
Jones, J. K. N., 23, 51, 116, 118, 129, 141, 142, 144, 149, 155, 156, 158, 159, 164, 167-169, 197, 213
Jones, W. G. M., 218
Jones, W. O., 159

K

Karrer, 66, 75, 104, 106, 112, 114, 190
Kenner, 7
Kenyon, 163
Kerr, 113
Kiessling, 136
Kiliani, 3, 6, 12, 14
Kiss, 17
Kitchen, 144
Klages, 98, 214
Klein, 96
Kluyver, 198

Knauf, 66
Knight, 7
Knoop, 211
Knorr, 60, 61
Koehler, 82
Kohn, 20
Königs, 60, 61
Kostanecki, 174
Kraemer, 125
Krajnc, 133
Kreider, 157
Kuehl, 75
Kuhn, W., 108

L

Lake, 72, 75
Lange, 5
Laurent, 195
Law, 92
Learner, 40, 53, 119
Lefèvre, 152
Leitch, 80, 83, 180
Leuchs, 74
Levene, 21, 64, 74, 81, 84, 157, 187
Levi, 206
Lewis, 16
Liebig, 170, 180
Link, 156
Liu, 211
Loach, 86
Lobry de Bruyn, 16
Loew, 23
Long, 83, 84, 86
Lorand, 132
Lowry, 27, 31, 46
Luckett, 156
Lunde, 218
Lyne, 210
Lythgoe, 187

M

McCloskey, 87, 97
McCready, 116, 117, 118, 133, 137, 152, 208
McDonald, 119, 211
McGettrick, 208
Machemer, 110, 111
Maclay, 152
Macpherson, 30
Malaprade, 4
Mark, 109, 216
Marker, 184
Martin, 168
Maurer, 78, 91
Mayer, 75
Menzies, 50
Meyer, 109, 112, 116, 118, 133, 135 144, 145, 147, 216, 219
Micheel, 48
Mikeska, 64
Miller, E. J., 35, 38, 53
Miller, I. L., 207
Misch, 109
Momber, 12
Monier-Williams, 105
Montgomery, 194
Morris, C. T., 117
Morris, D. L., 117
Mozingo, 75
Müller, 199
Muskat, 50

N

Nägeli, 114
Nagai, 95, 107
Neale, 129
Nef, 17, 33
Neuberg, 8
Neumann, 125
Nicholson, 93
Nickerson, 5
Nicolet, 200
Niemann, 98
Nishida, 112
Noelting, 133
Norberg, 168

O

Oakley, 147
Odier, 219
Öy, 218
Ohle, 68, 72
Oldham, 54, 67, 98
Oliver, 148, 149
Onuki, 102
Oppenauer, 203
Ott, 131
Owen, 68, 125

P

Pacsu, 47, 63, 88, 89
Palmer, 160
Partridge, 168
Paul, 39
Peat, 38, 66, 71, 72, 75, 80, 125, 137, 144, 146, 148, 149, 206, 214, 218
Peitzner, 211
Percival, E. E., 49, 217
Percival, E. G. V., 30, 48, 49, 52, 54, 56, 68, 107, 114, 115, 117-120, 122, 128, 129, 149, 201, 203, 210, 217, 218
Pigman, 43
Piloty, 19
Plant, J. H. G., 83
Plant, M. M. T., 116
Polanyi, 108
Porter, 56

Posternak, 196, 197, 198
Potter, 99
Prins, 118
Prochownick, 46
Pryzbytek, 20
Purdie, 32, 34, 39, 92
Purves, 34, 68
Pyle, 97

R

Rabaté, 90
Rabe, 62
Raistrick, 213
Rauch, 97
Rauchalles, 94
Record, 112, 120
Reeder, 5
Reeves, 162
Reichstein, 203
Reynolds, R. J. W., 88
Rice, 154
Richter, 87
Richtmyer, 69, 101, 193, 194
Ritchie, 128
Robertson, A., 171, 179
Robertson, G. J., 33, 70
Robinson, 177
Robiquet, 180
Rohrmann, 184
Rosanoff, 11
Rosenfeld, 69
Roudier, 144
Routledge, 98
Rüber, 30
Ruell, 86, 100
Ruff, 18, 20
Rundle, 50, 134, 135
Rutherford, 54

S

Soltzberg, 194
Sands, 164
Sätre, 149
Schiedt, 78
Schlubach, 46, 61, 91, 94, 98, 211
Schlüchterer, 163, 206
Schmidt, E., 129
Schmidt, G., 136
Schoch, 113
Schotte, 63
Sessler, 152
Severson, 113
Sheffield, 192
Shinn, 200
Siegrist, 219
Schneider, G. G., 156, 158
Schneider, W. S., 183
Silberkweit, 215
Simon, 27
Sinh, 211
Siwoloboff, 192
Smeykal, 50, 55
Smirnoff, 66
Smith, 56, 68, 76, 95, 97, 118, 152, 156, 165, 166, 201, 203, 204
Sobotka, 81
Somerville, 217
Sookne, 129
Soutar, 105
Sowden, 15, 65, 99, 108
Speakman, 161
Sponsler, 109
Stacey, 56, 76, 95, 97, 152, 154, 162, 163, 203, 206, 213, 219
Staudinger, 124
Steel, 39
Strauss, 172
Suckfüll, 48
Sutherland, 138
Svedberg, 125, 158
Swenson, 152
Szent Györgyi, 201

T

Tanret, 26, 27, 53, 66, 196
Thiel, 68
Thomas, 107
Thompson, 17
Thomson, 218
Tipson, 187, 209
Tobie, 5
Todd, 177, 187, 188
Tollens, 5, 25, 152
Tóth, 105, 106, 208, 216

V

v. Vargha, 47

W

Wadman, 169
Walder, 213
Waters, 171, 179
Webb, 60, 114
Weerman, 18
Wertheim, 116, 133
Westgarth, 38, 93
Widmer, 106
Wiggins, 71, 72, 76, 192, 194
Wilkinson, 116
Willstätter, 176
Winkler, 170
Winterstein, 197
Wöhler, 170, 180
Wohl, 12, 17, 20
Wolfrom, 16, 17, 47, 98, 108, 136, 207
Woodhouse, 105

Woolgar, 116
Woolvin, 63
Wrede, 183
Wylam, 85

Y

Yackel, 163
Young, E. G., 154
Young, F. G., 147
Young, G. T., 116, 139, 140, 141, 142, 143

Z

Zach, 68, 78
Zechmeister, 105, 106, 208, 216
Zeisel, 51
Zemplén, 17, 66, 81, 82, 84, 88, 97
Zervas, 152, 215

INDEX

A
Acetaldehyde, preparation of ethylidene compounds, 58
Acetobacter suboxidans, on *meso*inositol, 198
Acetobacter xylinum, action on mannitol and sorbitol, 193
  preparation of cellulose, 105
Acetobromoglucose, 61
  disaccharide synthesis, 62
  glycals, preparation, 63
  β-glucosan, preparation, 66
  glycoseens, preparation, 64
Acetohalogenose, 60
Acetone, cyanhydrin, aglucone of Linamarin, 182
  di*iso*propylidene derivatives, 54
Acetonitroglucose, 61
Acetophenone, β-glucosidoxy ω-O-tetraacetyl 4-acetoxy-, 177
Acetylation, 53
Acetyl bromide, degradation of methylated glycogen, 117
Acorns, oak bark, source of *d*-quercitol, 199
Acorn starch, 144
Acrolein dibromide, 23
α-Acrose, 23, 24
Adenine, 188
  in purine glycoside, 186
Adenosine, 188
  from adenylic acid, 186
    triphosphate, 188
    triphosphate, polysaccharide synthesis, 138
Adenylic acid, from muscle, 186
    3-phosphate, of yeast, 188
    5-phosphate, of muscle, 188
Adonis vernalis, source of adonitol, 191
Adonitol, 21, 191
  from *adonis vernalis*, 191
  preparation from D-ribose, 21
  natural occurrence, 21
  riboflavin, 189
Adrenal cortex, source of ascorbic acid, 201
Aesculetin, 174
Aesculin, 174
Aesculus hippocastanum, source of aesculin, 174
Agar-agar, 218
Aglucone, definition, 170
Aglycone, *see* Aglucone
Alanine, in periodate oxidation of 6-deoxy sugars, 201
Alcoholic fermentation, hydrogen carrier, 189
Aldehyde condensations, 58
Aldehydo-glucose, 2,3,4,5,6-pentaacetyl-, 47
Aldehydo-sugars, 47, 48
  derivatives, 47, 48
Aldobiuronic acids, 154
Aldohexose, definition, 2
  isomeric forms, 21
Aldonic acids, epimerisation, 15
Aldose to ketose transformation, 8, 16, 17, 78
Algae, source of erythritol, 191
Alginate, calcium, 159, 161
  fibres, 161
  textiles, 161
Alginic acid, 151, 158
  formula, 159
  molecular weight, 161
  properties, 161
  salts, 161
  X-ray, 160
Alizarin from ruberythric acid, 173
Allose, configuration, 21
  preparation from D-ribose, 21
D(+)-Allose formula, 13
Alloxan, riboflavin synthesis, 190
D-Allulose (D-psicose), 22
Allyl*iso*thiocyanate from sinigrin, 183
Almond tree gum, 167
Altrose, configuration, 21
  conversion to D-talomucic acid, 21
  preparation from D-ribose, 21
    3-amino- (epiglucosamine), 75, 78
D(−)-Altrose formula, 13
Amino-sugars, 74
Ammonia (liquid) to facilitate methylation, 50
Ammoniacal silver nitrate, paper chromatography, 169
Amygdalin, 170, 180
  formula, 180
  source of gentiobiose, 84
D-Amygdalin, heptaacetate, 181

D-Amygdalinamide, heptaacetate, 181
DL-Amygdalinamide, 181
  heptaacetate, 181
Amygdalinate, ethyl DL-heptaacetyl-, 181
β-Amylase, 144, 145, 147
  action on starches, 113, 114
  hydrolysis of amylose, 113
  hydrolysis of glycogen, 117, 147
  hydrolysis of starch dextrins, 145
Amylolysis, 144
Amylopectin, 113, 138
  chain length, various varieties, 141
  enzymic synthesis, 146
  formula from Meyer, 145
  general properties, 113
  laminated structure, 139
  separation from amylose, 113
Amylose, 113, 133
  amylopectin separation, 113
  crystalline, 113
  enzyme synthesis, 135, 146
  formula, 133
  general properties, 113
  helical arrangement, 134
  iodine complex, 134
  micelles, 135
  potentiometric titration, 141
  synthetic, 133
Anhydro sugars, 66, 218
  of ethylene oxide type, 69
Anhydro-, *For* anhydro- *derivatives see inverted entries under the name of the parent compound, e.g.* L-Galactose, 3,6-anhydro-, etc.
Anomers, 28
Anthocyanidins, 176
Anthocyanins, 176
Anthraquinone, 1,3-dihydroxy 2-methyl-3-glucoside-, 173
Antibodies, 162
Antigens, 162
Araban, 149
  associated pectic acid, 156
  associated pectin, 158
  decarboxylation theory, 158
Arabic acid, degraded, methylated, 165
  degraded, repeating unit, 165
  from gum arabic, 164
  suggested repeating unit, 166
Arabic gum, *see* Gums, arabic
Arabinose, configuration, 20
  from glucal, 63
D-(−)-Arabinose, formula, 13
  synthesis D-glucosamine, 74
  D-glucosido-, 81, 82
L-Arabinose, conversion to L-mannose, 14
  from gum arabic, 165
  in plant gums, 164
    acetobromo-, vicianose synthesis, 97
    2,5-dimethyl-, 166
    3-[galactosido]-, from gum arabic, 165
    2,3,4-trimethyl-, 33
    2,3,5-trimethyl-, 148, 166, 149
α-L-Arabinosides, emulsin hydrolysis, 170
Arabofuranose units, in pectic araban, 158
L-Arabofuranose, 2,5-dimethyl-, from methylated arabic acid, 166
    2,3,5-trimethyl-, 148, 149, 166
*Arabo*hexal, 65
*Arabo*hexosazone, 65
Arabonate, calcium, 19
Arabonic acid from glucoseen, (1,2), 64
D-Arabonolactone, 2,3,5-trimethyl-, 39
    2,3,4-trimethyl-, (δ), 40
γ-Arabonolactone, trimethyl-, from sucrose, 93
L-Arabonolactone, δ-trimethyl-, 33
6-[β-L-Arabopyranosido]-D-glucopyranose, 87
D-*Arabo*trihydroxyglutaric acid, 21
L-*Arabo*trihydroxyglutaric acid, 199
D-*Arabo*trimethoxyglutaric acid, 40
L-*Arabo*trimethoxyglutaric acid, 33-35
Arbutin, 171
    methyl-, 172
Arlitan, 194
Aromatic esters of sugars, 53
Ascent of the series, 14, 15
Ascent of aldoses, *see* Kiliani reaction
Ascorbic acid, 201-3
  from diacetone galactose, 56
  synthesis, 203
    dehydro-, 201, 203
    "imino"-, 204
    tetramethyl-, 202
    D-xylo-, 204
D-Ascorbic acid, 204
L-Ascorbic acid, 201-204
    6-deoxy-, 204
Ash tree, *see Fraxinus excelsior*, 174
Asparagosin, 211
Asparagus, source of asparagosin, 211
Avocado fruit, source of D-perseitol, 195

B

Bacterial polyuronides, 161
Bacterium gluconicum, preparation, 2-keto-gluconic acid, 154
B. mesentericus, production of fructosan, 209

B. subtilis, production of fructosan, 209
B. vulgatus, production of fructosan, 209
Barfoed's reagent, 79
Barley diastase, preparation maltose, 80
Barley leaves, 210
Bear berry (*Arbutus uva-ursi*), 171
Beet sugar, *see* Sucrose
Benzaldehyde, from amygdalin, 180
  4,6-benzylidene glucose, 58
  preparation mannitol tribenzylidene, 192
Benzene, 1-amino-2-carbethoxyamino-4,5-dimethyl-, riboflavin synthesis, 190
Benzoyl hydroquinone, arbutin synthesis, 171
Benzpyrone, 174
Bertrand's sorbose bacterium, 193
Beryllium alginates, 161
Betacoccus arabinosaceus, Orla-Jensen, dextran metabolism, 207
Bio-inosose, preparation DL-idosaccharic acid, 198
  from *meso*inositol, 198
Bios I, 197
  II, 197
Biotin, *see* Bios II
Black mustard, *see Sinapis nigra*
Boric acid, complex ions, 29
  conductivity D-glucoses, 29
  conductivity with *cis* a-glycols, 29
  mannitol, 192
Borneol glucuronide, 153
Brain tissue, natural glycosides, 171
Brewing, 80
Brigl's anhydride, 69, 98
Brown seaweeds, source alginic acid, 158
  source of fucoidin, 218
  source of D-mannitol, 191
Buckwheat, *see Fagopyrum esculentum*, 175
n-Butanol, separation amylose and amylopectin, 113
Butyramide, dimethoxy-hydroxy, from ascorbic acid, 202

C

Calcium, alginate, 159, 161
  D-arabonate, conversion to D-erythrose, 19
  salt of carrageenin, 217
  silicates, chromatography, 167
Californian pine, *see Pinus lambertiana*
Camphor, glucuronic acid detoxication, 153
Cane sugar, *see* sucrose
n-Caproic acid, 2-methyl-, from fructose, 6
Capsular polyuronide, 163
Carbohydrate, definition of, 1
Carbonates, 58
Carbonyl chloride, sugar carbonates, 58
Cardiac glucosides, 184
  cymarose, 185
Carrageenin, 217
  salts of, 217
Cartilage, 74, 76
" Celite ", for chromatography, 167
Cellobial hexaacetate, 95, 96
Cellobionate, methyl octamethyl-, from cellobiose, 83
Cellobiose, 82
  formula, 83
  synthesis, 95
    heptamethyl chlorhydrin-, synthesis cellotriose, 107
    hexaacetyl-, 95, 96
    octaacetyl-, 82
    octaacetyl-, chromatographic separation, 167
    octaacetyl-, from cellulose, 106
    octaacetyl-, from lichenin, 112
    octaacetyl-, synthesis, 95
    potassium hydroxide compound, 128
epi-Cellobiose, 1-bromo-heptaacetyl-, 95
    octaacetyl-, 95, 96
Cellobiuronic acid, 155
  from Pneumococcus type III, 162
  from Pneumococcus type VIII, 163
    tetramethyl-, from *Rhizobium* polyuronide, 163
Cellodextrin acetates, from cellulose, 111
Cellotetraose, from cellulose, 106
Cellotriose, from cellulose, 106
  synthesis, 107
Cellulose, 104, 107, 108, 123
  bacterial, 105
  carboxyl group, 129
  cellobiose, preparation, 82
  conductometric titration for chain length, 129
  configurational formula, 108, 111
  "cuprammonium" solution, molecular weight determinations, 125
  degradation during methylation, 126
  dyestuff penetration, 132
  hydrogen bonding, 130-132
  hydrolysis, 105-108
  methylation in nitrogen, 125-127
  micelle, microfibril, 131
  molecular weight, 123

  molecular weight by osmotic pressure, 125
  molecular weight by ultracentrifuge, 125
  molecular weight of various specimens, 125
  molecular weight by viscosity measurements, 124
  net-work bridges of Hess, 127-128
  rate of hydrolysis, 108
  raw material of industry, 104
  solubility, 131
  unit cell, 109
  van der Waal's forces, 130-132
  viscose rayon, 131
  X-ray, 108-110
  benzyl ethers softening points, 132
  derivatives, solubility, 130
    potassium hydroxide, 128
    triacetyl-, 105
    triacetyl-, solubility, 131-132
    trimethyl-, 105
    trimethyl-, hydrolysis, 110
    xanthate, 132
Cerebronic acid, 185
Cerebrosides, 171, 185
Cetraria Islandica, *see* Iceland Moss
Chain length, colorimetric determination, 133
Cheirolin, 183
Cherry tree gum, 167
Chitin, 74, 215, 217
  in crustaceans, 103
  fungal, 215
Chitinase, hydrolysis chitin, 215
Chitobionic acid, 216
Chitobiose, octaacetyl-, from chitin, 215
Chitosamine, 74-76
  natural occurrence, 74
    αβ-, hydrochloride, 74
Chitosaminic acid, 74
Chitose, 75
Chitotriose, hendecaacetyl-, from chitin, 216
Chloral hydrate, separation amylose and amylopectin, 113
Chondroitin sulphate, 76, 218, 219
  glucuronic acid in, 151
  structure, 218
Chondrosamine, 76, 78
  natural occurrence, 74
    N-acetyl-, 218
Chondrus crispus, reported source 2-ketogluconic acid, 154
  polysaccharide, 217
Chromatographic separation, lichenin, structural studies, 112
  cellobiose synthesis, 95
    hydrolysed, oxidised starch, 144
    hydrolysed, oxidised xylan, 150
Chromatography, 167
  blind, 167
  paper, 168
  *iso*sucrose synthesis, 98
Chrysophanein, 173
Chrysophanic acid, 173
Cinchonine salts, uronic acid identification, 151
Citrus pectin, 157
Coconut palm, *see under Cocos nucifera*
Cocos nucifera, source of scyllitol, 199
Coenzyme I, 188
  II, 189
Colorimetric determination of chain length, 133
Complex ions with boric acid, 29
Compositae, source of inulin, 118
Coniferin, 173
Coniferyl alcohol β-D-glucoside, 173
Copper sulphate, preparation of di-*iso*propylidene derivatives, 54
  osotriazoles, 6
Cori ester, 136
  synthesis, 136
Cornea of eye, source of hyaluronic acid, 218
Couch grass, source of triticin, 211
Coumarin, 7,β-D-glucoside, [7,8-dihydroxy-], 174
Crab shells, source of chitin, 215
Cranberry, source of idaein, 177
Cruciferae family, source of mustard oil glycosides, 183
Crustacea, 74, 103, 215
Cuprammonium hydroxide, dissolution of cellulose, 131
Cyanidin, 176
  from quercetin, 176
    3-β-D-galactoside, 177
Cyanin, 176
    3,5-β-diglucoside, 176
    3′-methylether, 177
Cyanogenetic glycosides, 179
  source of vicianose, 87
Cyanohydrins, 14
  cyanogenetic glycosides, 180
Cyclohexanes, hexahydroxy-, 195
  pentahydroxy-, 199
*Cyclo*pentenophenanthrene, glycosides, 184
    methyl-, 184
Cymarose, cardiac glucosides, 185, 199
Cytidine, 186
  synthesis, 187
Cytidylic acid, 187
Cytosine, from cytidine, 186
  from nucleosides, 186

D

Damson gum, 167
  glucuronic acid in, 155
  2-[β-D-glucuronosido]-D-mannose in, 156
Daphne, source of daphnin, 174
Daphnin, 174
Deacetylation, 82
Deadly nightshade, source of solanidine, 185
Decarboxylation theory, araban, 158
  galactan, 158
Dehydrogenation, sapogenin, 184
Delphinidin, formula, 177
  3′,5′-dimethyl ether-, 3,5-β-diglucopyranoside, 177
  3′, 5′, 7-trimethyl ether-, 3,5-β-diglucopyranoside, 177
Deoxy sugars, 54, 199
  6-, estimation, 200
  6-, periodic acid oxidation, 200
Deoxy-, *For* deoxy- *derivatives see inverted entries under the name of the parent compound*, e.g. Glucose, 2-deoxy-,
Descent of the aldose series, 17, 18
Desoxy sugars, *see* deoxy
Detoxication, 153
  mechanism, glucuronic acid, 152
  mechanism, glycosides, 171
Dextrans, 206
  molecular weight, 207
Dextrins, 145, 147
" Dextro " sugars, 11
Diabetes, action of rutin, 175
  phloridzin, 173
Diacetone-, *For* diacetone *derivatives see inverted entries under the name of the parent compound*, e.g. Galactose diacetone,
Dianhydrides, *see under parent compound*, e.g. β-D-Talose 2,3-1,6-dianhydro-,
Diazomethane, methylation of ascorbic acid, 202
  preparation of ketoses, 17
Dibenzoates of mannitol, 192
Dibenzyldiethylammonium hydroxide, dissolution of cellulose, 131
Dicarbonates, *see under parent compound*, e.g. Glucofuranose dicarbonate,
2,6-Diethoxypyrimidine, cytidine synthesis, 187
Diethylamine, preparation of glycoseens, 64
Digitalis spp., source of cardiac glucosides, 185
Digitalose, 185
Digitonin, 184
Digitoxigenin from digitoxin, 185
Digitoxin, 185
Digitoxose, 185
Digitoxose, from digitoxin, 185
3,5,-β-Diglucopyranoside, delphinidin 3′,5′-dimethyl ether, 177
  delphinidin 3′,5′,7-trimethyl ether, 177
3,5-β-Diglucoside of cyanin, 176
Diglycollate, distrontium D′D′-oxy*bis* (D-hydroxy-methyl)-, 91
  strontium D′-methoxy D-hydroxymethyl,- 41
  strontium L′-methoxy D-hydroxymethyl-, 41
Diglycollic acid, L′-oxy-D-methylene-, 67
Diglycollic aldehyde, D′-methoxy-, 41
  D′D′-oxy*bis*(D-hydroxymethyl)-, 91
  L′-oxy-D-methylene-, from β-glucosan, 67
Dihydroxyacetone, synthesis of hexoses, 23
Di*iso*propylidene derivatives, 56
" Dimedone ", 5,6-dimethyl cyclohexane, 1,3-dione, 4
L(+)-Dimethoxysuccinamide from ascorbic acid, 202
D(−)-Dimethoxysuccinic acid, 39
i-Dimethoxysuccinic acid from alginic acid, 159
L(+)-Dimethoxysuccinic acid, 38
  from ascorbic acid, 202
  from maltose, 80
  from polygalacturonic acid, 157
Dinucleotide, coenzyme I, 189
Disaccharides, 79
  definition, 1
  distinguishing test, 79
  enzymic synthesis, 98
  with 1,4-linkages, 80
  with 1,6-linkages, 84
  mutarotation, 79
  non-reducing, 90
  synthesis, 62, 94
Disappearing thread technique, 161
Dog fish liver and cartilage, source of scyllitol, 199
Douglas fir, 88
Dulcitol, 191, 194, 195
  source of D- and L- galactose, 212

E

Edible mussel, *see Mytilus edulis*
Egg plum gum, 167
  source of glucuronic acid, 151
  source of 6-[β-D-glucuronosido]-D-galactose, 155
Elder (common), *see Sambucus niger*

Emulsin, 158
  action on cellobiose, 28, 83
  action on gentianose, 99
  action on gentiobiose, 84
  action on β-methylglucoside, 27
  action on raffinose, 100
  amygdalin hydrolysis, 180
  cellobiose hydrolysis, 83
  galactosides hydrolysis, 170
  plant glycosides hydrolysis, 170
  synthesis cellobiose, gentiobiose, 99
End group assay, 110, 116-118, 158, 210
Enzymes, glycoside hydrolysis, 170
Enzymic synthesis, amylopectin, amylose, 146
*Epi*glucosamine, *see* Altrose 3-amino-
Epimeric sugars, 15
Epimerisation, *see* Aldonic acids
Erythrin, 191
Erythritol, 191
  (*meso*)-, 20
D-Erythrose, 20
  formula, 13
    2-[β-D-galactosido)-, 84
    D-glucosido-, 81, 82
Esparto grass, 121
Estimation of, aldoses, 111
  6-deoxy sugars, 200
  formaldehyde, 4
  sucrose, 92
  sugar, refractive indices, 167
  sugar, micro-reducing, 168
  uronic acid, 152
Ethereal sulphate, in polysaccharides, 217
Ethers, triphenyl methyl-, 52
Ethylene oxide, ring fission, 73
  ring formation, 73
    anhydrides, 69
α-Ethylglucofuranoside, 59
    2,3,-diacetate 5,6-carbonate, 59
β-Ethylglucofuranoside, 59
    2,3,-diacetate 5,6-carbonate, 59
Ethylidene compounds, 58
Ethyl DL-mandelate, 181
Ethyl mercaptan, reagent for determining molecular size cellulose, 108
Euxanthic acid, 175
  glucuronic acid detoxication, 153
Euxanthone, 175

F

Fagopyrum esculentum, source of rutin, 175
Fischer's, convention for formulae, 9, 11
  synthesis of sugars, 23
Flavanone, 7-rutinoside, 5,7,3′-trihydroxy-4′-methoxy-, 175
Flavone, 174
    3,5,7,3′,4′-pentahydroxy-, 175
    [5,7,3′-trihydroxy-6′,4′,5′,-trimethoxy *iso*,] 7-glucoside, 175
Flavylium, 3,5,di[β-glucosidoxy]-7,4′-dihydroxy-, chloride-, 177
Flax plants, source of linamarin, 182
Florida clay, for chromatography, 167
Floridoside, 170
Formaldehyde, preparation of methylene compounds, 58
Formulae, projection, Fischer's convention, 9
Frangulin, 173
Fraxetin, 174
Fraxin, 174
Fraxinus excelsior, source of fraxin, 174
Fructofuranose, dimethyl-, from irisin, 211
    tetramethyl-, 38, 93, 102
    tetramethyl-, from irisin, 211
    tetramethyl-, from inulin, 120
    tetramethyl-, from levan, 209
    tetramethyl-, from raffinose, 100
    trimethyl-, 119, 209, 210
β-Fructofuranosidase, on sucrose, 94
α-D-Fructofuranoside, β-D-glucopyranosido-, 98
β-D-Fructofuranoside, α-D-glucopyranosido-, 93, 94
Fructopyranose, phenylosazone, ring formula, 49
    α- and β- diacetones-, 55, 56
    tetramethyl-, 40
β-D-Fructopyranose, ring formula, 45
Fructosamine, 78
Fructosazone, 7
Fructosazone, trimethyl-, from methylated inulin, 119
Fructose, configuration, 22
  existence of acyclic form, 46
  from 1-aminoglucose, 78
  pentahydroxyketone, 6
  from sucrose, 92
  sweetness, 2
    1-amino-, 78
    α-diacetone-, 55, 56
    β-diacetone-, 55, 56
    β-diacetone-, 2-ketogluconic acid preparation, 154
    dimethyl-, from polyfructosans, 211
  osazone formation from, 7
    p-nitrophenylhydrazone, 101
    tetraacetate, from sucrose octaacetate, 98
    tetramethyl-, 39, 93, 100, 102

    tetramethyl-, rotations, 92
    trimethyl-, from levan, 209, 210
    3,4,6-trimethyl-, from inulin, 119
D-Fructose, configuration, 22
  from inulin, 119
  from mannitol, 193
    3-[α-D-glucopyranosido]-, 88
    phenylosazone, 49
L-Fructose from L-arabonic acid, 17
  synthesis of sugars, 24
Fructoside, *see* Methylfructo-furanoside and -pyranoside
Fructuronic acid, 154
Fucoidin, 218
  source of fucose, 200
Fucose, derivatives from galactose derivatives, 54
    diacetone-, 57
L-Fucose, 200, 218
Fucoside, *see* Methylfucoside, etc.
Fungal chitin, 215
Fungi, 74, 90
  source of erythritol, 191
Furanose ring, 46
Furanoses, furanosides, 44
Furfural, 5-methyl-, from 6-deoxy sugars, 200

G

Galactan, associated pectic acid, 156
  decarboxylation theory, 158
  pectin, 213
  pectin, repeating unit, 213
Galactocarolose, 213
D-Galactofuranose, 2,3,5,6-tetramethyl-, from galactocarolose, 213
Galactogen snail, 212
L-Galactomethylose, 200
L-Galactonamide, 57
D-Galactonic acid, conversion to D-lyxose, 18
L-Galactonic acid, 57
δ-D-Galactonolactone, 2,3,4,6-tetramethyl-, 36, 38
γ-D-Galactonolactone, 36
Galactopyranose, 44
    2,4-dimethyl-, from methylated arabic acid, 166
    tetramethyl-, 83, 84, 100, 102, 212, 213
    tetramethyl-, from arabic acid, 166
    tetramethyl-, from gum arabic, 165
    tetramethyl-, from melibiose, 86
    tetramethyl-, from slippery elm mucilage, 164
    2,3,4-trimethyl-, 102, 165
    2,3,6-trimethyl-, 213
    2,4,6-trimethyl-, 217
D-Galactopyranose, in carrageenin, 217
  ring formula, 44
L-Galactopyranose, 212
6-[α-D-Galactopyranosido]-D-glucopyranose, 86, 97
4-[β-D Galactopyranosido]-D-glucopyranose, 84
6-[β-D-Galactopyranosido]-D-glucose 86, 97
D-Galactosamine, 74
    N-acetyl-, 218
β-Galactosan, 76
  from lactose, 66
  from β-phenylgalactoside, 66
Galactose, configuration, 22
  from lactose, 83
  from melibiose, 86
  from solanine, 185
    acetobromo-, lactose synthesis, 96
    acetobromo-, melibiose synthesis, 97
    2-amino-, hydrochloride-, 76, 77
    3,6-anhydro-, 68
  derivatives, conversion to fucose, 54
    1,2-3,4-diacetone-, 55
    diacetone-, galacturonic acid preparation, 153
    diacetone 6-tosyl-, 57
    2,4-dimethyl-, from galactogen, 212
    2,4-dimethyl-, from gum arabic, 165
    2,6-dimethyl-, 54
    2-methane-sulphonyl 1,6-anhydro-, from β-galactosan, 76
    6-methyl-, 56
    3,4-monoacetone 1,6-anhydro- from β-galactosan, 76
    1,2,3,4-tetraacetyl-, galacturonic acid preparation, 153
    tetraacetyl 6-iodo-, diethyl mercaptal, 48
    2,3,4,6-tetramethyl-, 36, 83, 84 100, 102, 212, 213
    2,3,4-trimethyl-, 102, 165
    2,3,6-trimethyl-, from slippery elm mucilage, 164
    2,4,6-trimethyl-, 164, 217
D(+)-Galactose, formula, 13
  from mucilage of *Ulmus fulva* bark, 164
    2-amino- 1,6-anhydro-, 76, 77
    3-[D-galactosido]-, from degraded arabic acid, 165
    6-[β-D-glucuronosido]-, 155, 165
    6-[β-D-glucuronosido]-, from gum arabic, 164

    6-[β-D-glucuronosido]-hexamethyl-, from degraded arabic acid, 165
    2-methyl 1,6-anhydro-, from 2,3-1,6-dianhydro-β-D-talose, 77
    phenylosazone from chondrosamine, 76
    2,3,4,6-tetramethyl-, from pectin galactan, 213
    2,3,6-trimethyl-, from pectin galactan, 213
    2,4,6-trimethyl-, from agar-agar, 217
L-Galactose, from galactogen, 212
    3,6-anhydro, from agar-agar, 218
    6-deoxy-, 200
D-Galactoseptanose, 2,3,4,5-tetraacetate, 48
β-Galactosidases, hydrolysis lactose, 83
α-D-Galactosides, emulsin hydrolysis, 170
  glycerol, 170
  see Methylgalactoside, etc.
3-β-D-Galactoside, cyanidin-, 177
β-D-Galactosides, emulsin hydrolysis, 170
3-[Galactosido]-L-arabinose, from gum arabic, 165
2-[β-D-Galactosido]-D-erythrose, from lactose, 84
3-[D-Galactosido]-D-galactose, 165
6-[α-D-Galactosido]-D-glucopyranose, 97
D-Galacturonic acid, 57, 151, 153
  natural sources, 151
  in pectic acid, 156
  in plantain and linseed families and slippery elm mucilage, 164
    diacetone-, 57, 153
    2,3-dimethyl-, from slippery elm mucilage, 164
    2,3,4-trimethyl-, 164
2-[α-D-Galacturonosido]-L-rhamnose, 155, 164
Gastric mucosa, source of mucoitin sulphate, 218
Gaultherase, 172
Gaultheria procumbens, source of gaultherin, 172
Gaultherin, 172
Gelidium, spp. source of agar-agar, 217
Gentian (yellow), source of gentianose, 84
Gentianose, 84
  formula, 99, 100
Gentiobiose, 84
  from amygdalin, 180
  synthesis, 96
    acetobromo-, 181
    octaacetyl-, chromatographic separation, 167
    octaacetyl-, formula, 97
β-Gentiobioside, D(−)mandelonitrile, 180
  see Methylgentiobioside, etc.
Geranium, *see* Pelargonium
Gigartina stellata, source of carrageenin, 217
Gitonin, 184
Glucal, 63, 64, 65
    triacetate, 63
    trimethyl-, 63
Glucitol, 1,3,5-triazoyl 2,4,6-trimethyl-, 112
    1,4,5-triazoyl 2,3,6-trimethyl-, 112
Glucocheirolin, 183
2-Glucodesose, *see* Glucose 2-deoxy
Glucodesoside, 64
Glucofuranolactone, 2,3,5,6-tetramethyl-, 81, 83, 114, 115
Glucofuranose, 1,2-5,6-diacetone-, 55, 57
    dicarbonate, 58
    1,2-monoacetone-, 57
    1,2-monoacetone 5,6-anhydro-, 72
    1,2-monoacetone 3,5-benzylidene-, 58
    1,2-monoacetone 5,6-benzylidene-, 58
    1,2-monoacetone-, 5,6-carbonate, 59
Glucoheptonic acid, 4
Gluconic acid, from glucose, 3
    2,3,4,5-tetramethyl-, 86
    2,3,5,6-tetramethyl-, 81, 83, 84, 102
    trimethyl 2-keto-, 38, 39
δ-D-Gluconolactone, from β-D-glucose, 43
    tetramethyl-, 35, 38
γ-Gluconolactone, 14, 36
    tetramethyl-, 81, 83, 114, 115
D-Glucopyranose, 45
  relative reactivity of hydroxyl groups, 51
  ring formula, 44
    1,6-anhydro-, 95
    6-[β-L-arabopyranosido]-, 87
    1-chloro-2,3,4-triacetyl-, 97
    1,6-dibromo-2,3,4-triacetyl-, 96
    4-[β-D-galactopyranosido]-, 84
    6-[α-D-galactopyranosido]-, 86, 87
    6-[α-D-galactosido]-, 97
    6-[β-galactosido]-, 97
    αα′-glucopyranosido-, 97
    1-[α-glucopyranosido]-, 91

4-[α-D-glucopyranosido]-, 81
4-[β-D-glucopyranosido]-, 83
6-[β-D-glucopyranosido]-, 86
pentabenzoyl-, 53
1-phosphate, 136
1-phosphate, synthesis, 136
6-[β-L-rhamnosido]-, 88
6-[β-L-rhamnosido]-, heptaacetate, 88
4-sodio-1,2,3,6-tetraacetyl-, 95
1,2,3,4-tetraacetyl-, 97
1,2,3,6-tetraacetyl-, 95
2,3,4,6-tetraacetyl-, 98
1,2,3,4-tetraacetyl 6-trityl-, 96
tetramethyl-, 35, 51, 83, 85, 91, 92, 102, 107, 110, 114, 115, 140, 163, 207
2,3,4-trimethyl-, 84-86, 100, 207
2,3,6-trimethyl-, 80, 83, 102, 105-107, 110, 112, 114, 116, 162, 163
2,4,6-trimethyl-, 207
6-[β-D-xylopyranosido]-, 87
D-Glucopyranoside, 3,5-di-, delphinidin 3′5′-dimethyl ether, 177
3,5-di-, delphinidin 3′,5′,7-trimethyl ether, 177
6-β-, 6,7-dihydroxy-coumarin-, 174
α-, 1-[α-D-glucopyranosido]-, 91
αα′-glucopyranosido-, 97
3-[α-D-glucopyranosido]-D-fructofuranosido-, 101
α-D-Glucopyranosido-, 3-, D-fructose, 88
4-, D-glucopyranose, 81
1-,α-D-glucopyranoside, 91
3-α-D-Glucopyranosido-α-D-fructofuranosido-α-D-glucopyranoside, 101
4, D-mannitol nonaacetate, 89
6, D-glucose. octaacetate, 207
4-, D-mannose, 88
α-L-sorbofuranoside, 99
xyloketofuranoside, 99
β-D-Glucopyranosido-, 4-, D-glucopyranose, 83
6-, D-glucopyranose, 86
2-, D-glucose, 90
4-, D-mannose octaacetate-, 95, 96
D-Glucosamine, 74
from chitin, 215
N-acetyl-, 76
N-acetyl-, from chitin, 215
N-acetyl-, from hyaluronic acid sulphate, 218
N-acetyl-, from mucoitin sulphate, 218
hydrochloride, X-ray, 75
L-Glucosamine, N-methyl-, 75
Glucosaminic acid, 216
β-Glucosan, cellobiose synthesis, 95
from β-phenylglucosides, 66
properties, 66, 67
from starch, from lactose, 66
synthesis, 66
*Laevo*glucosan from starch, 66
Glucosazone, 8
from D-glucosamine, 74
reduction to 1-aminofructose, 78
DL-Glucosazone, synthesis of hexoses, 24
Glucose, from amygdalin, 180
from dimethylamino-methylglucosaminide, 75
from gaultherin, 172
from gentiobiose, 84
isomeric glucoses, 26, 27
from lactose, 83
from melibiose, 86
a pentahydroxy-aldehyde, 3
periodic acid oxidation, 5
in plant gums, 164
from sarsasaponin, 184
from sinigrin, 183
from solanine, 185
from sucrose, 92, 93
sweetness, 2
acetobromo-, 60-64
acetobromo-, arbutin synthesis, 171
acetobromo-, cellobiose synthesis, 95
acetobromo-, Cori ester synthesis, 136
acetobromo-, gentiobiose synthesis, 96
acetobromo-, indican synthesis, 179
acetobromo-, linamarin synthesis, 182
acetobromo-, pelargonin synthesis, 177
acetobromo-, phlorin synthesis, 172
acetobromo-, *iso*trehalose synthesis, 97
acetonitro-, 61
6-N-alanino-, 72
6-amino-, 78
6-alkyl-, from 1,2-monoacetone-5,6-anhydroglucofuranose, 72
1,2-anhydro-, preparation, 69
1,2-anhydro-, triacetate, *see* Brigl's anhydride
1,6-anhydro-, 95
3,6-anhydro-, 68
4,6-benzylidene-, 58
1-chloro-2,3,4-triacetyl-, 97

  cyanhydrins, 3
  2-deoxy-, 64, 199
  diacetone-, 54
  diacetone-, methylation, 50
  1,6-dibromo-, triacetate, preparation glucoseen-(5,6), 65
  1,6-dibromo-, triacetate, gentiobiose synthesis, 96
  di*iso*propylidene-, 54
Glucose, dimethyl-, from amylopectin, 139
  dimethyl-, from cellulose, 127
  2,3-dimethyl-, from *Rhizobium* polyuronide, 163
  2,3-dimethyl-, from starch, 143
  2,4-dimethyl-, 162
  dimethyl mercaptal-, 47
  6-diphenylamino-, 72
  4,6-ethylidene-, 58
  6-α-D-glucopyranosido-, octaacetate, 207
  2-methyl-, from cellulose, 129
  2- and 6-methyl-, from lichenin, 129
  3-methyl-, 56
  5-methyl-, 46
  6-methyl-, from cellobiose, 128
  methylene-, 58
  1,2-monoacetone 3,5-benzylidene-, glucuronic acid preparation, 152
  1,2-monoacetone-, 5,6-carbonate, 58
  oxime, 3, 18
  pentaacetate, 47, 53, 60
  pentaacetates, isomeric, 26
  6-phenyl-, 72
  phenylhydrazone, 3, 7
  phenylosazone, 7
  phenylosazone, ring structure of, 48
  1-phosphate, 136
  1-phosphate, from glycogen, 136
  1-phosphate, glycogen synthesis, 148
  1-phosphate, ketofuranosides syntheses, 99
  1-phosphate, starch synthesis, 136
  6-phosphate, 138, 188
  6-phthalimido-, 72
  propylidene (di-*iso*), 54
  4-sodio-, tetraacetate, 95
  1,2,3,4-tetraacetyl-, gentiobiose synthesis, 96, 97
  1,2,3,4-tetraacetyl-, glucuronic acid preparation, 152
  1,2,3,4-tetraacetyl-, vicianose and primeverose synthesis, 97
  1,2,3,6-tetraacetyl, 95
  2,3,4,6-tetraacetyl-, 61
  2,3,4,6-tetraacetyl-, *iso*sucrose synthesis, 98
  2,3,4,6-tetraacetyl-, *iso*trehalose synthesis, 98
  tetramethyl-, 35, 38, 51, 85, 107, 114-116
  2,3,4,6-tetramethyl-, from cellobiose, 83
  tetramethyl-, from cellulose, 110
  tetramethyl-, chromatographic separation, 168
  2,3,4,6-tetramethyl-, conversion to 2,3,4,6-tetramethyl mannose, 16
  2,3,4,6-tetramethyl-, from maltose, 80
  2,3,4,6-tetramethyl-, from *Rhizobium* polyuronide, 163
  2,3,4,6-tetramethyl-, from disaggregated starch, 140
  2,3,4,6-tetramethyl-, from sucrose, 92
  2,3,4,6-tetramethyl-, from trehalose, 91
  3-tosyl diacetone-, 70
  2-trichloracetyl-, 69
  trimethyl-, chromatographic separation, 168
  2,3,4-trimethyl-, from gentiobiose, 84
  2,3,4-trimethyl-, from β-glucosan, 67
  2,3,4-trimethyl-, from *Leuconostoc* dextran, 206
  2,3,4-trimethyl-, from melibiose, 86
  2,3,4-trimethyl-, from raffinose, 100
  2,3,6-trimethyl-, from cellobiose, 83
  2,3,6-trimethyl-, from cellulose, 105-107, 110
  2,3,6-trimethyl-, from cellobiuronic acid, 162
  2,3,6-trimethyl-, from lactose, 83
  2,3,6-trimethyl-, from lichenin, 112
  2,3,6-trimethyl-, from maltose, 80
  2,3,6-trimethyl-, from *Rhizobium* polyuronide, 163
  2,3,6-trimethyl-, from stachyose, 102
  2,3,6-trimethyl-, from starch, 114, 116
  2,4,6-trimethyl-, from laminarin, 207
  2,4,6-trimethyl-, from yeast glucan, 208

    - 3,5,6-trimethyl-, from diacetone glucose, 57
    - 6-trityl-, gentiobiose synthesis, 96
- D-Glucose, 11, 119
  - conductivity in boric acid, 29
  - configuration, 22
  - conversion to L-gulose, 19
  - formula, 13
  - from laminarin, 207
  - from maltose, 80
  - from trehalose, 91
    - 2-amino-, 74
    - 1,2-anhydro-, 69
    - 6-[β-D-galactopyranosido]-, 86, 97
    - 2-[β-D-glucopyranosido]-, 90
    - 4-[β-D-glucuronosido]-, 155
    - β-pentaacetyl-, 53
    - 6-trityl-, 52
- L-Glucose, 11
  - from L-arabinose, 15
    - 1-phosphate, 138
- D-Glucoseen, (1,2), 64
    - tetraacetyl-, 64
- Glucoseen, (5,6), 65
- α-Glucosidase, on amylopectin, 145
  - preparation of maltose, 80
- Glucosides, *see under name of specific glucoside*, e.g. α-Methylglucoside, cardiac glucoside, etc.
- Glucosido-, trimethylammonium bromide tetraacetyl-, preparation β-glucosan, 66
- D-Glucosido-, D-arabinose, from maltose oxime, 81, 82
    - D-erythrose, 81, 82
    - D-mannose, 88, 89
    - phloroglucinaldehyde, 177
- 3-β-Glucosidoxyindole, 179
    - 2-carboxylic acid, 179
- β-Glucosidoxyindole, methyl-3-O-tetraacetate 2-carboxylate, 179
    - 3,O-tetraacetate, 179
- Glucosone, 8
  - from lactose, 83
    - 2-ketogluconic acid prep., 154
- Glucurone, from glucuronic acid, 152
- Glucuronic acid, in chrondroitin, 218
  - detoxication mechanism, 152
  - from heparin, 151
  - in hyaluronic acid sulphate, 218
  - natural sources, 151
  - preparation, 152
  - from plant gums, 164
    - 2,3,-dimethyl-, from methyl arabic acid, 166
    - 2,3-dimethyl-, from *Rhizobium* polyuronide, 163
    - methoxy-, from plant gums, 164
    - 2,3,4-trimethyl-, from arabic acid 166
    - 2,3,4-trimethyl-, from gum arabic, 165
- D-Glucuronic acid, 19, 58, 151, 152
- 6-[β-D-Glucuronosido]- D-galactose, 155, 165
- 4-[β-D-Glucuronosido]- D-glucose, 155
- 2-[β-D-Glucuronosido]- D-mannose, 156
- α-Glucosyl, 1-bromide, tetraacetyl-, 60
    - 1-chloride, tetraacetyl-, 61
- β-Glucosyl, 1-nitro-, tetraacetyl-, 61
- Glutaric acid, *For glutaric acid derivatives see under arabo-* and *xylo-*glutaric
- Glycals, 63
- Glyceraldehyde (glycerose), 9, 10, 11, 12
  - Fischer's synthesis of hexoses, 23
- D-Glyceric acid, from periodate oxidations, 42
  - from trehalose, 91
- Glycerol, α-D-galactoside, 170
- Glycerose, 9-13
  - transformation to tartaric acids, 12
- D-Glycerose, 42
  - configuration, 11
  - relationship to, D-trioses, D-tetroses pentoses, hexoses, 13
- Glycogen, 117, 147
  - in animals, 103
  - from edible mussel, 147
  - chain formula, 117
  - chain lengths of various, 118
  - enzymic synthesis, 148
  - laminated structure, 147
  - multiple branched structure, 147
  - natural occurrence, 117
- α-Glycols, *see* under boric acid
  - *cis*, oxidation, 5
  - *trans*, oxidation, 5
- Glycosans, 66
- Glycoseens, 64
- Glycoside, detoxication mechanism, 171
  - enzymes, 170
  - nitrogen, 171, 186
  - ring structure, 40
- Glycosides, *see under specific glycoside*, e.g. Cyanogenetic glycosides, Methylglycosides, etc.
- " Glyptal " resins, 192
- *Gracilaria confervoides*, source of agar-agar, 217
- Graminin, 211
- " Grenzdextrin ", I, 145
  - II, 145
  - III, 145
- Guanine, purine glycoside, 186

D-Gulonic acid, transformation to D-idonic acid, 15
L-Gulonic acid α-keto-, *see* 2-Keto-L-gulonic acid, 204
D-Gulose, 22
  formula, 13
L-Gulose, configuration, 22
  preparation from D-glucose, 19
  from L-xylose, 15
Guloside, *see* Methylguloside, etc.
Gum, arabic, 164
  source of glucuronic acid, 151
  source of 6-[β-D-glucuronosido]-D-galactose, 155
  cherry tree, 167
  levan, 209, 210
  plant, 164
  sterculia setigera, 23
  tragacanth, source of L-fucose, 200
Gymnema sylvestre, source of L-quercitol, 199

H

Haptens, specific polysaccharides, 162
Helicin, 172
  benzoyl, 172
Helinus ovatus, source of scyllitol, 199
Helix pomatia, 212
  enzyme for cellulose hydrolysis, 104
  source of galactogen, 212
Hemicelluloses, 164
Heparin, 151, 218
Heptahydric alcohols, 195
Heptose, definition, 2
n-Heptylic acid, 3
Hesperidin, 88, 175
Hevea brasiliensis, source of linamarin, 182
Hexabenzoates of mannitol, 192
Hexahydric alcohols, naturally occurring, 191
Hexanitrate of mannitol, 192
Hexokinase, polysaccharide synthesis, 138
Hexosamines, 74
Hexoses, as six-membered rings, 34, 40, 43
  definition, 2
    6-deoxy-, periodic acid oxidation, 200
  general properties, 2
  synthesis with dihydroxyacetone, 23
Hexuronic acid, *see* Ascorbic acid, 201
Hirsutin, 177
Honey, 2
Horsechestnut tree, *see* under *Aesculus hippocastanum*, 174
Hudson's, convention for anomeric sugars, 28
  lactone rule, 36, 37
Hyaluronic acid sulphate, 74, 218
Hydrazine, α-methylphenyl-, detection of ketoses, 8
Hydrazodicarbonamide, 18
Hydriodic acid, Zeisel estimation, 51
Hydrocyanic acid, cyanogenetic glucosides, 180
  glucosides, plant protection, 171
Hydrofuranol ring, 67
Hydrogenation, catalytic, D-glucose, 193
  sucrose, 191
Hydrogen carrier, coenzyme I, 188
Hydroquinone β-D-glucoside, 171
Hydroxyanthraquinoneglycosides, 173
p-Hydroxybenzoic acid from pelargonidin, 177
p-Hydroxybenzyl *iso*thiocyanate, 183
α-Hydroxy *iso*butyric ester, synthesis of linamarin, 182
Hydroxycoumaringlycosides, 174
Hydroxyflavoneglucosides, 174
α-Hydroxy groups, diagnostic test, 18
Hydroxyl groups, reactivity, 51

I

Iceland Moss, 112
Idaein, 177
L-Iditol, 191
  natural source, 193
DL-Idosaccharic acid from *bioinosose*, 198
Idose, configuration, 22
D-Idose, formula, 13
  from D-gulonic acid, 15
    3-acetamido-1,6-anhydro-, diacetate, 77
Immunology, 162
Indican, 179
  synthesis, 179
Indigo, 179
Indigofera, source of indican, 179
Indigotin, 179
Indole, 3-β-glucosidoxy-, 179
    3-β-glucosidoxy-, 2-carboxylic acid, 179
    β-glucosidoxy-,methyl,3-O-tetraacetate 2-carboxylate, 179
    β-glucosidoxy-, 3-O-tetraacetate, 179
    3-hydroxy,- 179
    methyl 3-hydroxy-, 2-carboxylate, 179
Indoxyl, *see* under Indole 3-hydroxy-
Inositols, 195
  deoxy-, 199
d-Inositol, monomethyl ether, 196
l-Inositol, 196

  methyl ether, 196
*meso*Inositol, 197
  natural sources, 197
  diphosphate, 197
  hexaphosphate, 197
  monophosphate, 197
  tetraphosphate, 197
racemic Inositol, 196
Inosose, *bio*-, from *meso*inositol, 198
Interrelationships of the sugars, 19
Inulin, 118
  chain formula, 119
  glucose in, 119
  molecular weight, 120
  natural sources, 118
  rate of hydrolysis, 119
Invertase, on gentianose, 84
  on raffinose, 86, 100
  on sucrose, 92
Iodine, substances giving blue colour, 135
Iodo compounds, from toluenesulphonates, 54
2-Iodohexane, from sorbitol, glucose, 3
Iridin, 175
Iris florentina, 175
Irisin, 211
Isomeric forms, aldohexoses, 21
  ketohexoses, 22
Ivory nut palm, source of mannan, 214

K

Ketofuranoside, synthesis, by phosphorylase, 99
2-Ketogluconic acid, preparation, 154
2-Ketogluconamide, 2,3,4,6-tetramethyl-, 38
    2,3,4,6-tetramethyl-, from inulin, 119
    2,3,4,6-tetramethyl-, from levan, 210
    2,3,4,6-tetramethyl-, from sucrose, 93
5-Keto-L-gulonic acid, 154
2-Keto-L-gulonic acid, ascorbic acid synthesis, 204, 205
Ketohexose, definition, 2
  isomeric, 22
2-Ketosaccharamide, dimethyl-, from levan, 209
2-Ketosaccharic acid, dimethyl-, from levan, 209
Ketose, detection, 8
  preparation, 16, 17
  ring structure, 38
Ketouronic acids, 154
Kiliani reaction, 14

L

Lactarius volemus, source of D-volemitol, 195
Lactobionic acid, 84
Lactobionolactone from lactose, 84
Lactoflavin, *see* Riboflavin
γ-Lactones, rate of hydrolysis, 34
δ-Lactones, 43
  rate of hydrolysis, 33
Lactosazone, 83
Lactose, 83
  formula, 84
  synthesis, 96
Lactoside, *see* Methyllactoside, etc.
Lactosone, 83
" Laevo " sugars, 11
Laevulose, *see* Fructose
Laminated structure, 139
Laminaria cloustoni, source of laminarin, 207
Laminaribiose, 90
Laminarin, 207, 208
  estimation of chain length, 208
  source of laminaribiose, 90
  periodic acid oxidation, 208
Larch, 88
Lead tetraacetate oxidation, 5
Leuconostoc dextran, 207
Leuconostoc, *dextranicum*, dextran, 206
  mesenteroides, dextran, 206
Levan, 209
  end group essay, 210
    triacetyl-, 209
    trimethyl-, 209
Lichenin, 112
  chain length, 112
  from Iceland moss, 112
    potassium hydroxide-, 129
Lichens, source of erythritol, 191
Lignoceric acid, in cerebrosides, 185
Lime tree, 88
Limit dextrins, oxidised, hydrolysed products, chromatographic separation, 144
Linamarin, (Phaseolunatin), 182
Linseed family, source of galacturonic acid, 164
Lobry de Bruyn transformation, 16
Lobster shells, source of chitin, 215
Lyxose, configuration, 20
D-(−)-Lyxose, formula, 13
L-Lyxose, from diacetone D-galactose, 57

M

Madagascar manna, source of dulcitol, 195
Madder root, source of alizarin glycosides, 173

- source of primeverose, 87

- Magnesium silicates, hydrated, for chromatography, 167
- Maltal, 88
  - hexaacetate, 89
- Maltase, action on α-methylglucoside, 25, 27
  - maltose hydrolysis, 80
  - sucrose hydrolysis, 94
  - synthesis maltose, 99
- Maltobionate, methyl octamethyl-, from glycogen, 117
    - methyl octamethyl-, from starch, 114, 115
- Maltobionic acid, from maltose, 80
- Maltobionic ester, methyl octamethyl-, from maltose, 80, 81
- Maltobionolactone, rate of hydrolysis, 81
- Maltosazone, 79
- Maltose, 79, 80
  - from amylose, 113
    - heptaacetate, from starch, 114
    - octaacetate, 88
    - oxime, 81
- Maltoside, *see* Methylmaltoside, etc.
- Maltosido-1-bromide heptaacetate, 88, 114
- Malvin, 177
- Mandelonitrile glucoside, 180, 182
- D[−]-Mandelonitrile β-vicianoside, 182
- L[+]-Mandelonitrile β-D-glucoside, 182
- DL-Mandelonitrile β-D-glucoside, 182
- Mango leaves, glucuronic acid detoxication, 153
- Manna of ash tree, source of D-mannitol, 191
- Mannan, " A ", 214
  - chain length, 214
  - " B ", 214
- Mannan yeast, 214
- β-Mannide, 192
- *iso*-Mannide, 192, 193
- *neo*-Mannide, 192, 193
- Manninotriose, from stachyose, 101
- D-Mannitan, 192, 193
- Mannitol, 192
  - periodic acid oxidation, 4
    - 1,4-anhydride, 192
    - 3,6-anhydride, 192
    - 1,4-3,6-dianhydride, 192
    - 1,5-3,6-dianhydride, 192
    - dibenzoates, 192
    - dichlorhydrin, β-mannide preparation, 192
    - hexabenzoates, 192
    - hexanitrate, 192
    - tetrabenzoates, 192
    - 1,2-3,4-5,6-triacetone-, 192
    - tribenzylidene-, 192
- DL-Mannitol, synthesis of sugars, 24
- D-Mannitol, 191
  - from *laminaria digitata*, 191
    - 1,5-anhydride, 193
    - 4-[α-D-glucopyranosido]-, nonacetate, 88, 89
- Mannofuranose, 2,3-5,6-diacetone-, 55
    - 2,3-5,6-dicarbonate, 60
- D-Manno-D-*gala*heptitol, 195
- L-Mannomethylose, 200
- DL-Mannonic acid, synthesis of sugars 24
- D-Mannonic acid from D-mannitol, 193
- γ-D-Mannonolactone, tetramethyl-, 60
- β-D-Mannopyranose, 1,6-anhydro 2,3-monoacetone-, synthesis cellobiose, 95
- Mannosaccharic acid, dimethyl-, 159
- D-Mannosaccharic acid, from mannitol, 193
- Mannosaccharodilactone, D-mannuronic acid preparation, 154
- Mannosan, from ivory nut, 66
  - from β-phenylmannoside, 66
- β-D-Mannosan, cellobiose synthesis, 95
- Mannose, 63
  - in plant gums, 164
  - sweetness, 2
    - 2-amino-, 74
    - 3,4-dimethyl-, from yeast mannan, 214
    - 2,3,6-trimethyl-, from mannan, 214
    - 3,4,6-trimethyl-, 30, 62
    - 3,4,6-trimethyl-, from mannan, 214
- D-Mannose, from benzylidene methylglucosaminide, 75
  - configuration, 22
  - formula, 13
  - mannans, 214
  - from mannitol, 193
    - 1,6-anhydro-2,3-monoacetone-, 95
    - 4 [β-D-glucopyranosido]-, octaacetate, 95, 96
    - 4-[α-D-glucosido]-, 88, 89
    - 2-[β-D-glucuronosido]-, 156
    - 4-[β-D-tetraacetylglucopyranosido]-, synthesis cellobiose, 95
    - 2,3,4-trimethyl-, from mannan, 214
    - 2,4,6-trimethyl-, from mannan, 214
    - 3,4,6-trimethyl-, 214
    - 3,4,6-trimethyl-, conductivity in boric acid, 30, 62

L-Mannose, from L-arabinose, 15
  6-deoxy-, 200
α-D-Mannosides, emulsin hydrolysis, 170
  see Methylmannoside, etc.
D-Manno-D-*gala*-heptitol, 195
D-Manno-D-*talo*heptitol, 195
" Manucol ", emulsifier, 161
Mannuronic acid, from alginic acid, 151
  2,3-dimethyl-, 159
D-Mannuronic acid, 151, 154
  *meso*dimethoxysuccinic acid, from alginic acid, 159
Melezitose, formula, 101
  natural occurrence, 100
  periodate oxidation, 101
  source of turanose, 88
Melibionate, methyl octamethyl-, 86
Melibiose, 86
  formula, 87
  synthesis, 97
Melibioside, *see* Methylmelibioside, etc.
Mercuric salts, removal of thioalkyl groups from mercaptals, 47, 48
Methanesulphonates, preparation of ethylene oxide ring compounds, 70
α-Methylalloside, 2,3-anhydro-4,6-benzylidene-, 70
β-Methylalloside, 2,6-dimethyl 3,4-anhydro-, 72
  4,6-dimethyl 2,3-anhydro-, 71
β-Methylaltropyranoside 3-amino-4,6-dimethyl-, 75, 76
Methylaltrosaminide, 3-amino-, 78
α-Methylaltroside, 2,3,4-tribenzoyl-, 69
  2,6-anhydro-, 69
β-Methylaltroside, 4-6-dimethyl-, 71
  3,4,6-trimethyl-, 72, 75, 76
β-Methylarabofuranoside, ring formula, 44
  trimethyl-, chromatographic separation, 168
Methyl-L-arabopyranosides, 33
Methylated sugars, Zeisel estimation, 51
Methylation, 33, 50
  methyl iodide and silver oxide, 33, 50
  methyl sulphate and sodium hydroxide, 33, 50
Methylcellobioside, heptamethyl-, 83
β-Methylcellotrioside, decamethyl-, 107
Methylchondrosaminide, N-acetyl 3,4,6-trimethyl-, from chondroitin, 219
Methylene compounds, 58
β-Methylfructofuranosides, action of taka-invertase, 94
"γ"-Methylfructofuranoside, ring structure, 38
β-Methylfructopyranoside, ring structure, 39
α-Methyl-L-fucoside, monotrityl ether, 52
Methylgalactopyranosides, 35
Methylgalactoseptanoside, 48
α-Methylgalactoside, 3,6-anhydro-, 68
  6-tosyl-, preparation 3,6-anhydro derivatives, 68
β-Methylgalactoside, 2,6-dimethyl-, 54
Methylgalacturonosides, 2,3-dimethyl methyl ester-, 157
Methylgentiobioside, heptamethyl-, 85
α-Methylglucofuranoside, 59
  ring formula, 44
    3,6-anhydro-, from 3,6-anhydroglucopyranoside, 68, 69
α- and β-Methylglucopyranosides, 34
α-Methylglucopyranoside, 51
  4,6-ethylidene-, 58
  tetramethyl-, 51
β-Methylglucopyranoside 2-amino 4,6-dimethyl-, 75, 76
  2-amino 3,4,6-trimethyl-, 75, 76
  4,6-benzylidene-, 58
  6-nitrate, preparation 3,6-anhydro derivatives, 68
  6-sulphate, preparation 3,6-anhydro derivatives, 68
  tetraacetate, 61
  6-tosyl-, preparation 3,6-anhydro derivatives, 68
  2,3,4-trimethyl-, 52, 53
  2,3,4-trimethyl 6-trityl-, 52
Methylglucosaminide, benzylidene-, 75
  N-acetyl trimethyl-, 76
Methylglucosides, 25-27
  chromatographic separation, 168
  6-bromotriacetyl-, in 6-amino glucose preparation, 78
    2,3-dimethyl-, from a dextran, 206
    4,6-dimethyl-, 71
    dimethyl-, from starch, 140
    potassium hydroxide-, 52
    2,3,4,6-tetramethyl-, 35, 206
    2,3,4-trimethyl-, 51, 206, 207
    2,4,6-trimethyl-, 72, 75, 76
    2,3,6-trimethyl-, 72, 140
α-Methylglucoside, 51
  action of maltase, 25, 27
    2-benzoyl 3-tosyl 4,6-benzylidene-, 70

  2-tosyl 3-benzoyl 4,6-benzylidene-, 70
β-Methylglucoside, 26-27, 34
 hydrolysis by emulsin, *see* Emulsin
 relationship to isomeric glucose, 27
  3,6-anhydro-, 68
  6-bromhydrin-, 67
Methyl D-glucuronoside, 2,3,4-trimethyl-, 219
γ-Methylglycosides, 32, 37
β-Methylguloside, 2,4,6-trimethyl-, 72
Methyllactoside, heptamethyl-, 83
Methylmaltoside, heptamethyl-, 80
α-Methylmannofuranoside, 60
α-D-Methylmannopyranoside, ring formula, 44
Methylmannoside, D-mannuronic acid preparation, 154
 from glucal, 63
  2,3-anhydro-, 78
  2,3-anhydro 4,6-benzylidene-, 70
  monoacetate, to give orthoester, 62
  2,3,4-triacetate, 154
α-Methylmannoside, 2,3,4-tribenzoyl-6-*p*-toluenesulphonate-, 54
β-Methylmannoside 2,3-anhydro-4,6-dimethyl-, 72, 75
"γ"-Methylmannoside, 60
Methylmelibioside, heptamethyl-, 86
Methylphenylhydrazine, detection of ketoses, 8
Methylrhamnoside, monoacetate, 62
Methylsalicylate, 172
γ-Methylsulphone propyl*iso*thiocyanate, 183
Methylxylobionic ester, hexamethyl-, 121, 122
Methylxylofuranoside, 38
Methylxylopyranoside, 34
  trimethyl-, chromatographic separation, 168
Methylxyloside, 2,3-dimethyl-, 52
  3,4-dimethyl-, 52
  2,4-dimethyl-, 52
Micelles, amylose, 135
 see Cellulose
Microfibril, *see* Cellulose
Micro-reducing power, sugar estimation, 168
Mistletoe berries, 196
Molecular refraction and configuration, 31
Monosaccharides, definition, 1
 Haworth's nomenclature, 43
 relative configuration, 10
Morphine mannonates, 24
Mountain ash, source of sorbitol, 193
Mucic acid, 22
 from *l*-inositol, 197
 from quercitol, 199
Mucilages, 164
Mucoitin sulphate, 218
Mucopolysaccharides, 74, 218
Mucoproteins, 74
Mustard oil glycosides, 62, 183
Mustard, black, 183
 white, 183
Mutarotation, 26, 27, 31
 equilibrium between cyclic and open chain forms, 46
 α- and β-D-glucoses in boric acid, 29
Myrosinase, action on glucocheirolin, 183
 action on sinigrin, 183
Mytilus edulis, edible mussel, 147

N

Naphthalene, 1,2,5-trimethyl-, 184
Naphthoresorcin, uronic acid identification, 151
Nasal septa, 76, 218
Nasturtium, source of β-phenylethyl *iso*thiocyanate, 183
Naturally occurring hexoses, 20, 23
Nerve tissue, natural glycosides, 171
Nicotinic acid amide, coenzyme I, 189
Nitrogen glycosides, 171, 186
Nitromethane, ascent of series, 15
Nomenclature, sugar derivatives, 64, 65
Nucleic acid, 186
Nucleoproteins, 171, 186
Nucleosides, 186
 source of cytosine, 186
 synthesis, 62

O

Oak, American, *see Quercus tinctoria*
Oil of, bitter almonds, 170
 wintergreen, 172
Oligosaccharides, 79
 definition, 2
Optical isomers, 9
Orcin, *see* Toluene 3,5-dihydroxy-
Orris root, *see Iris florentina*
Orsellinic acid, source erythritol, 191
Orthoesters, 62
Osazones, 6
  p-bromophenyl-, 8
  tolyl-, 8
Osmotic pressure, molecular weight determination, 120, 125, 147, 148, 158, 161
Osone, 8
Osotriazoles, 6
Ovomucoid, 74, 215
Oxamide, from ascorbic acid, 202
Oxidation, bromine, dimethyl mannuronic acid, 159
 bromine water, maltose, 80

  dinitrogen tetroxide, 163
  fuming nitric acid, *meso*inositol, 197
  of glucose, 3
  lead tetraacetate, 5
  nitric acid, mannitol, 193
  nitric acid, populin, 172
  nitric acid, salicin, 172
  nitric acid, L-sorbose, 205
  nitric acid, trimethyl glucose, 80
  periodate, 40, 69
  periodate, cellulose, 129
  periodate, chondroitin sulphate, 219
  periodate, β-glucosan, 67
  periodate, glycogen, 147
  periodate, melezitose, 101
  periodate, pectic acid, 157
  periodate, starches, 141
  periodate, trehalose, 91
  periodate, xylan, 149
  periodate (potassium) on 1,4-linked polysaccharide, 129
  periodic acid, amylopectin, 144
  periodic acid, 6-deoxyhexose, 200
  periodic acid, glucose, 5
  periodic acid, laminarin, 208
  periodic acid, mannosaccharic acid, dimethyl-, 159
  periodic acid, methylated lichenin hydrolysates, 112
  periodic acid, methylhexosides, 41
  periodic acid, methylpentosides, 41
  periodic acid, yeast glucan, 209
Oxide rings, 2, 5, 31, 32
Oxonium salts, anthocyanins, 176
Ozone, preparation arabinose, 63

P

Paraldehyde, preparation ethylidene glucose, 58
Parillin, *see* Sarsasaponin
Pea phosphorylase, 136, 146
Pectic acid, 151, 156
  end group assay, 158
  source of galacturonic acid, 151
  methylation, 157
  molecular weight, 158
  natural sources, 156
  periodate oxidation, 157
  X-ray analysis, 160
Pectin, pectic acid, 156
  with xylan, 149
    galactan, 213
Pelargonin, 177
  synthesis, 177, 178
Pelargonium, 177
Penicillium charlesii, G. Smith, 213
Pentaphan ring, 67
Pentoses, as six-membered rings, 34, 40
  configuration, 20
  definition, 2
    methyl-, 199
Pentosides, oxidation with periodic acid, 41
P-enzyme, 146
Peonin, 177
Peony, source of peonin, 177
Perbenzoic acid, action on glucal, 63
  synthesis cellobiose, 95
Periodate oxidation, *see* Oxidation periodate
Periodic acid oxidation, *see* Oxidation, periodic acid
D-Perseitol, 194, 195
Phaeophyceæ, brown seaweeds, 158
Phaseolunatin, *see* Linamarin
Phenanthrene *cyclo*penteno glycosides, 184
Phenolic glycosides, 171
Phenol propionic acid, 172
Phenols, from glycosides, 171
Phenyl azobenzoyl ester, sugar derivative, in chromatography, 112, 167
β-Phenyl ethyl *iso*thiocyanate, 183
β-Phenylgalactoside, 66
β-Phenylglucoside, 62
  glucosan preparation, 66
Phenylhydrazonium ion, 7
Phenylmannoside, 66
Phenylosazones, 6
Phlein, 211
Phloretin from phloridzin, 172
Phloridzin, 172
Phlorin, 172
Phloroglucide, 172
Phloroglucin aldehyde, 177
    2-O-β-[glucosido]-, 177
Phloroglucinol, 195
  estimation of 6-deoxy sugars, 200
  from pelargonidin, 177
    β-D-glucoside, 172
Phosphatase, 197
Phosphate bond, " energy rich ", 138
Phosphates, preparation, 56
Phosphoglucomutase, 138
Phosphoric acid, dissolution cellulose, 131
  on nucleotides, 186
Phosphorolysis, 136
Phosphorus pentachloride, preparation 1,2-anhydroglucose, 69
Phosphorylase, 136, 148
  ketofuranoside synthesis, 99
Photosynthesis, 1
Phytic acid, 197
Phytin, 197
Pine tree sap, source coniferin, 173
Pinitol, *see* *d*-Inositol monomethyl ether, 196

Pinus lambertiana, 196
"Piuiri", 153
Plantain, source D-galacturonic acid, 164
Plant gums, *see* Gums, plant, 164
Pneumococcus, Type III, 162
  formula polysaccharide, 162
  Type VIII, 162
Poa trivalis, source of fructosan, 210
Polarimetric observation, 43, 108
Polyfructosans, 209
  with 1,2-links, 211
  with 1,6-links, 211
Polygala amara, source polygalitol, 194
Polygalactans, 211
Polygalacturonic acid, methylated, 157
Polygalitol, 193
  from sorbitol, 194
Polyglucosans, 206
Polyhomologous series, 124
Polyhydric alcohols, 191
Polymannosans, 214
Polysaccharide, definition, 2
  general formula, 103
  general properties, 103
  synthesis, 138
Polyuronides, 156
  bacterial, 161
  from cellulose, 163
  definition, 2
  from gums and mucilages, 164
  from *Rhizobium radicicolum*, 163
Poplar tree, 88
  source of populin, 172
Populin, 172
Potassium phenate, in preparation of glycosides, 62
Potassium salt of carrageenin, 217
Potatoes, source of solanine, 185
  phosphorylase, 136, 138, 146
Primary alcoholic groups, methylation, 52
Primula plants, source of primeverose, 87
Primeverose, 79, 87
  in gaultherin, 172
  from ruberythric acid, 173
  synthesis, 97
Propiophenone, 2,4,6-trihydroxy-β-[4-hydroxyphenyl]-, 172
*iso*Propylidene compounds, 54
Prulaurasin, 182
Prunasin, 182
Prunus, *spp.* source mandelonitrile glucoside, 182
Pseudoamylose, 146
Pseudomonas saccharophila, ketofuranoside synthesis, 99
D-Psicose, 22
Purgative drugs, 173
Purine glycosides, 186
Purines, nucleoside synthesis, 62
Purpurin, 173
Pyranose ring, 44
Pyranoses, pyranosides, 44
Pyrimidine glycoside, 186
Pyrus, *spp.* source of Arbutin, 171
Pyrylium salts, polyhydroxyphenyl, 176

Q

Q enzyme, amylopectin synthesis, 146
Quebrachitol, *see* *l*-Inositol methylether, 196
Quebracho bark, source *l*-inositol methyl ether, 196
Quercetin, 174
    3-rutinoside, *see* Rutin
Quercitols, 199
l-Quercitol, 199
Quercitrin, 174, 175
Quercus tinctoria, 174
Quinoline, arbutin synthesis, 171
  melibiose synthesis, 97
  orthoester preparation, 62

R

Raffinose, formula, 100
  source of levan, 209
  source of melibiose, 86
  natural sources, 100
    hendecamethyl-, 100
Red seaweeds, 217
Reduction by sugars, 2, 5
Reducing power, cellulose hydrolysis, 108
Refractive indices, sugar estimation, 167
Retrogradation of amylose, 113
L-Rhamnopyranose, 2,3,4-trimethyl-, 166
D-Rhamnose, preparation, 54
D-*iso*Rhamnose, gentiobiose synthesis, 96
L-Rhamnose, 200
  from gum arabic, 166
  from quercitrin, 174
  from sarsasaponin, 184
  from solanine, 185
  from *ulmus fulva* bark, 164
    acetobromo-, rutinose synthesis, 97
    3,4-dimethyl-, from slippery elm, 164
    2-[α-D-galacturonosido]-, 155, 164
    4-methyl-, 164
L-Rhamnoside frangula-emodin, 173
  see Methylrhamnoside, etc.

6-[β-L-Rhamnosido]- D-glucopyranose, 88
Rhizobium radicicolum polyuronide, 163
Rhodymenia palmata, 170
Rhubarb, source of chrysophanein, 173
  Chinese, source of frangula-emodin, 173
Ribamine, 2-carbethoxyamino-4,5-dimethyl phenyl-, riboflavin synthesis, 190
Ribitol, riboflavin, 189
9-[D-Ribityl]-6,7-dimethyl-isoalloxazine, 189
2-Ribodesose, *see* Ribose 2-deoxy
Riboflavin, 189
  synthesis, 190
Ribofuranose, in uridine and cytidine, 187
    tetraacetate, 187
    1,2,3-triacetate, 187
    1,2,3-triacetyl 5-trityl-, 187
3-[β-Ribofuranosido], 6-ethoxypyrimidine triacetate, 187
Ribose, configuration, 20, 21
  formula, 13
  in adenosine, 188
  preparation adonitol, 189
  riboflavin synthesis, 190
  in nucleotides, 186
    2-deoxy, 64, 186, 199
    2-deoxy, in nucleotides, 186
    2-deoxy, occurrence, 64
Ring formulae, 5
  6-membered in hexoses, 44
  β-D-glucopyranose, 44
Rosanoff convention, 11
Rotation of lactones, Hudson's rule, 36
Rowan tree, source sorbitol, 193
Rubber tree, see *Hevea brasiliensis*
Ruberythric acid, 173
  source of primeverose, 87
Rubiadin, 173
Ruff degradation, 18
Rutin, 88, 175
  prophylactic action, 175
Rutinose, 88
    heptaacetate, 97
7-Rutinoside, 5,7,3′-trihydroxy-4′-methoxyflavone-, 175
Rye, source of graminin, 211
  source of secalin, 211

S

Saccharic acids, 3, 11
    dimethyl-, from maltose, 80
    tetramethyl-, diamide, 86
    trimethyl-, from gentiobiose, 85
D-Saccharic acid, conversion to L-gulonic acid, 19
  from L-inositol, 196, 197
D- and L-Saccharic acids from *meso*-inositol, 198
Saccharinic acids, 17
D-Saccharolactone, 19
  D-glucuronic acid preparation, 152
Sachse " chair ", strainless, 160
Salicin, 172
  preparation β-glucosan, 66
Salicylic acid, 3,5-dinitro-, colorimetric chain length determination, 133
Saligenin β-D-glucoside, 172
Sambucus niger, source sambunigrin, 182
Sambunigrin, 182
Sapogenin, 184
Saponins, 184
Sapotalene, 184
Sarsaparilla root, 184
Sarsasapogenin, 184
Sarsasaponin, 184
Schiff's reagent, 5
Schweizer's reagent, 131, 215
Scurvy, 201
  action of rutin, 175
Scyllitol, 198, 199
Sea onion, source sinistrin, 211
Seaweeds, brown, *see* Brown seaweeds
  red, 217
Secalin, 211
Selenium, dehydrogenation sapogenin, 184
Senna plant, 196
Separation of sugar mixtures by chromatography, 167
Septanoses, 47, 48
Silver carbonate, action on acetobromo compounds, 61
  cellobiose synthesis, 95
Sinalbin, 183
Sinapinic acid, 183
Sinapin sulphate, 183
Sinapis nigra, 183
Sinistrin, 211
Slippery elm mucilage, 155, 164
Snail, edible, *see Helix pomatia*
  source of chitinase, 215
Sodium alginate, 161
Sodium bromide, addition compound sucrose, 94
Sodium chloride, addition compound sucrose, 94
Sodium hydroxide, action on cellulose, 131
Sodium hypoiodite, estimation aldoses, 111

Sodium methoxide, a deacetylating agent, 82
  hydrolysis to give ethylene oxide rings, 70
Sodium pectate, 160
Solanidine, 185
Solanine, 185
Sophora japonica, 90
Sophorose, 90
*iso*Sorbide, 194
Sorbitan, (1,4), 194
Sorbitan, (1,5), 194
Sorbitol, a hexahydric alcohol, 3
    1,5-anhydride, 194
    1,4-3,6-dianhydride, 194
    hexaacetate, 3
D-Sorbitol, 193
  synthesis ascorbic acid, 204
    3-[α-D-glucopyranosido]-,nonaacetate, 88
L-Sorbofuranose, diacetone-, 204
α-L-Sorbofuranoside, α-D-glucopyranosido-, 99
Sorbose, existence acyclic form, 46
D-Sorbose, configuration, 22
L-Sorbose, ascorbic acid synthesis, 204, 205
  enzyme disaccharide synthesis, 99
  natural source, 193
  preparation, 193
Sphingosine, in cerebrosides, 185
Stachyose, natural occurrence, 101, 102
    tetradecamethyl-, 102
Starch, 112
  acorn, 144
  banana, 142
  chain formula, 115
  chain length, 141
  chestnut, 141
  chromatographic separation, oxidised, hydrolysed, 144
  dextrins, 145
  disaggregation, 140
  general properties, 114
  hydrogen bonds, 141, 142
  hydrolysis products, separation, 144
  laminated structure, 139
  maltose source, 80
  methylated, kinetics of disaggregation, 141
  methylation in nitrogen, 140
  molecular weight, disaggregated, 140
  natural, chain length, 115
  periodate oxidation, 141
  rate of hydrolysis, 115
  rice, laminated structure, 140
  sago, separation hydrolysis products after oxidation, 144
  synthesis, by potato phosphorylase, 136
  synthesis, priming agents, 137
  synthetic, molecular weight and properties, 137
  union of neighbouring chains, 143
  waxy maize, 113
Sterculia setigera gum, 23
Stereoisomerism, 8-10
Streptomycin, 75
Strophanthus, spp., 185
Strychnine mannonates, 24
Styracitol, 192, 193
    6-tosyl 2,3,4-tribenzoyl-, 192
Styrax obassia, source styracitol, 193
Succinic acid, dimethoxy-, 38, 80, 159
Sucrose, 79, 91, 92, 94
  source of dextrans, 206
  enzyme synthesis, 99
  estimation polarimetrically, 92
  fructosan source, 209
  hydrogenation, 191
  mannitol and sorbitol source, 191
  primary product photosynthesis, 92
    heptamethyl-, 93
    octamethyl-, 92
*iso*Sucrose, 98
  synthetic, chromatography, 98
Sugar, anhydrides, 66
  beet, 92
Sugars, cyclic structure, 25
    deoxy-, 200
  α-, β-, forms, 31
  halohydrins, 60
  hydrazones, 48
  lactones, conversion to aldoses, 14
  D-series, 11
  L-series, 11
Sulphates, preparation, 56
Sulphonic esters, 53
Sulphur derivatives, 62
Syringin, 173

T

D-Tagatose, 20
D-Tagatose, configuration, 22
  from *Sterculia setigera*, 23
Taka-invertase, on sucrose, 94
D-Talitol, 1,5-anhydro-, 69
D-Talomucic acid, 21
  from *meso*inositol, 198
D-Talose, configuration, 22
  formula, 13
    2,3-1,6-dianhydro-, 76
    3,4-1,6-dianhydro-, 77
D-Tartaric acid from pectic acid, 157
*meso*Tartaric acid, 20
  from *meso*inositols, 197
Tendons, 74, 218
Tetrabenzoates of mannitol, 192

- Tetramethyl α-methylglucopyranoside, *see* α-Methylglucopyranoside, tetramethyl-
- Thallium, methylation, 51
  - methylation degraded pectic acid, 157
  - hydroxide, methylation, 51
- Thallous derivatives, use in methylation, 51
- Thiobarbituric acid, estimation 6-deoxy sugars, 200
- Thioglucose, from sinigrin, 183
- L-Threonamide, trimethyl-, 201, 202
- D-Threose, 20
  - formula, 13
  - oxidation to tartaric acid, 12
- Thymol, separation amylose and amylopectin, 113
- Timothy grass, 211
- Toad poisons, digitoxin glucoside, 185
- Toluene 3,5-dihydroxy, uronic acid identification, 151
- Toluene *p*-sulphonates, 54, 70
- Tolylosazones, 8
- Torsional effect of ring closure, 45
- "Trehala manna", 90
- Trehalose, 90, 91, 97
  - periodate oxidation, 91
  - synthesis, 98
    - octamethyl-, 91
- *iso*Trehalose, formula, 98
  - octaacetate, 97
- *neo*Trehalose, 97, 98
- Trimethylamine, preparation glucosan, 66
- Triphenylchloromethane, 52
- Triphenylmethyl ethers, *see* Ethers, triphenyl methyl-, 52
- Trisaccharides, definition, 1
- Triterpenoid nucleus, 184
- Triticin, 211
  - molecular weight, 211
- Trityl ethers, *see* Ethers triphenylmethyl-, 52
- "Tunicin", 103
- Turanose, 88, 90
  - isomeric octaacetates, 89, 90
  - keto form octaacetate, 88, 90
  - from melezitose, 100
    - orthoester, 63
    - phenylosazone, 90
- Turnips, 183
- Type III (*pneumococcus spp.* polysaccharide), 155, 162
  - VIII, 162

U

- *Ulmus fulva*, 164
- Umbilical cord, 218
- Uracil, from nucleosides, 186
  - from uridine, 186
- Uridine, 186
- Uridylic acid, 187
- Uronic acids, 151, 152
  - derivatives, 152
  - estimation, 152
  - identification, 151

V

- Vegetable ivory, *see* Ivory nut palm
- *Vicia angustifolia*, 87, 182
- Vicianin, 182
- Vicianose, 87
  - synthesis, 97
- β-Vicianoside D(−)mandelonitrile-, 182
- Viscose rayon, 131, 132
- Vitamin B_2, 189
- Vitamin C, 201
- D-Volemitol, 195

W

- Walden inversion, 43
  - acetobromo compounds, 61
  - amino sugars, 75
  - ethylene oxide anhydride, 69, 71
  - ethylene oxide ring formation, 73
  - ethylene oxide rings, 70
- Wallflower seeds, 183
- Weerman degradation, 18
- Wintergreen, oil of, 172
- Wohl descent of the aldose series, 17

X

- Xanthone, pigments, 175
    - 1,7-dihydroxy-, 7-glucuronoside, 175
- X-ray, alginic acid, 160
  - amylose, 134, 135
  - application to configuration of glycosides, 28
  - ascorbic acid, 203
  - cellulose, 108-110
  - chitin, 216
  - glycogen, synthetic, 148
  - pectic acid, 158, 160
  - sucrose, 94
- Xylan, 121, 148
  - chain formula, 121
  - formula, 149
  - laminated structure, 149
  - molecular weight, 148
  - natural sources, 121
  - oxidised hydrolysates, 150
  - periodate oxidation, 149
- Xyloketofuranoside, α-D-glucopyranosido-, 99
- D-Xyloketose, 17, 99

Xylonolactone, 2,3,4-trimethyl-, 34
    2,3,5-trimethyl-, 121, 122
β-D-Xylopyranose, ring formula, 45
Xylopyranose, trimethyl-, 122, 148
6-β-D-Xylopyranosido-D-glucopyranose, 87
L-Xylosazone, 56
Xylose, configuration, 20
  from gaultherin, 172
  units in plant gums, 164
    acetobromo-, 97
    1,2-3,5-diacetone-, 56
    2,3-dimethyl-, 121
    2-methyl-, 149
    2,3,4-trimethyl-, 16, 34, 121
    2,3,5-trimethyl-, 38
D-Xylose, 10
  conversion to D-xyloketose, 17
  formula, 13
  degradation to threose, 12
  from xylan, 121
L-Xylose, 2,4-benzylidene-, 15
Xyloside, *see* Methylxyloside, etc.
β-D-Xylosides, hydrolysis emulsin, 170
L-Xylosone, 56
  synthesis ascorbic acid, 204
*Xylo*trihydroxyglutaric acid, 21
i-*Xylo*trimethoxyglutaric acid, 34, 35
  from gentiobiose, 84, 85

Y

Yeast glucan, 208
  periodic acid oxidation, 209

Z

Zeisel estimation, 51
Zinc, preparation glycals, 63
Zinc chloride-hydrochloric acid, hydrolysing agent for cellulose, 107
  preparation benzylidene derivatives, 58